

Chapter 8

Micromechanical biosensors for virus detection

**Kutay Sagdic, Özgecan Erdem, Esma Derin, Saeedreza Zeibi Shirejini,
Yusuf Aslan, Suleyman Celik, Umit Celik and Fatih Inci**

The rapid identification of viruses has vital implications for managing health care, such as controlling disease at early stages and preventing the potential spread of disease. Conventional methods are, however, time-consuming and they often require expensive and sophisticated infrastructure that limits their use in point-of-care settings. Biosensors have attracted notable attention in the last decades, as they are central to applications that monitor many processes, and they offer portability and ease-of-use, enhancing their utility. Among these, micromechanical biosensors rely on small mechanical transducers to sense any vibrations that are precisely proportional to molecular bindings. In this chapter, we comprehensively summarize the field of micromechanical biosensors along with their fundamentals, fabrication, and fundamental characteristics along with pros and cons. Moreover, we mainly focus on how micromechanical biosensors can be tailored to detect viruses such as coronavirus, human immunodeficiency virus (HIV), hepatitis, noroviruses, Ebola, and Zika viruses that pose an overwhelming threat to global health.

8.1 Fundamentals of micromechanical biosensors and actuators

Biosensors reliant on micromechanical resonators are widely utilized to detect specific analytes in liquid, air, or *in vacuo*. These sensors include cantilevers and various other structures such as mechanical components that create acoustic waves and surface stress. In this section, we briefly explain the types and fundamentals of micromechanical biosensors.

8.1.1 Micro-nanomechanical sensors

The development of the atomic force microscope (AFM) began with the use of a cantilevered sharp tip to mechanically probe a surface [1]. In AFM, cantilevers are employed in either static mode or dynamic mode. In the contact mode, the surface is mapped by using the static deviation caused by the contact with the surface as a

feedback source. In the dynamic mode, the cantilever is driven at a frequency close to the resonance frequency, and the surface is therefore mapped using the cantilever vibration amplitude as a feedback source. Later, the flexibility of this technique was demonstrated by the development of several new sensing modes, for example, magnetic properties, capacitance, conductivity, or phase-based measurements.

Recently, micro- and nanometer-scale cantilevers have been studied as sensors using physical principles, which are similar to those found in AFM systems. As basically described above, these cantilevers are usually operated either in the static deflection mode, in which binding on one side of a cantilever leads to unbalanced surface stress, resulting in a measurable up or down deflection, or in the dynamic mode, in which binding on the cantilever increases the mass, and thus decreases the resonant frequency. Cantilever-based sensors offer highly versatile approaches using multiple modes, such as electrostatic, optical, mechanical, and electromagnetic strategies for actuating or sensing cantilever motion to detect chemicals, gases or biological entities.

With the development of microfabrication technologies, significant advances have been made in micro-electromechanical systems/nano-electromechanical systems (MEMS/NEMS)-based biosensor technologies in recent years. MEMS/NEMS cantilever-based sensor applications offer more portable, compact, ultrasensitive options and lower detection limits compared to standard approaches. In addition to their inexpensive features, they provide measurements in real-time, and with appropriate chemical functionalization, they can be employed to monitor minute changes in biological conditions and/or chemical reactions. Moreover, microfabricated devices can be designed with different sizes and shapes and formed into arrays with large numbers of elements. This flexibility of design and size has created the opportunity to combine such devices with microfluidics and miniaturized lab-on-a-chip formats. They are able to monitor small changes in signals caused by molecular/biological vibrations and can be operated with very small sample volumes. Moreover, the relevance of these platforms to the field of virus detection is immediate since they are able to detect biological targets at nanometer-scale [2]. High-frequency MEMS/NEMS platforms are being considered as a new direction in the field of sensing. In particular, the flexibility to adapt their surface properties using specific biomolecules is used to create chemically functionalized MEMS/NEMS cantilevers for biomolecular interaction studies. These devices also exhibit superior potential for multiplexed biosensor arrays due to their small physical dimensions and excellent sensitivity [3, 4]

8.1.2 Cantilever-based micromechanical biosensors

Considering a biosensing approach, the cantilever surface is first functionalized to create a bioreceptor layer, which has a high affinity for the target analytes. When a molecular interaction occurs between the bioreceptor layer and the target analytes, the cantilever surface energy changes in response to the binding of the target to the sensor's surface. As a result, the cantilever deviates statically. In a resonating cantilever biosensor, the resonance frequency alters according to the mass increase or decrease on the cantilever. Moreover, cantilever-based biological or chemical detection methods can be divided into static cantilever bending and resonating cantilever-based detection [5].

8.1.2.1 Static cantilevers

Static cantilevers measure bending or static deflection (static mode: DC mode), which can be induced by external forces, such as gravitational, magnetic, and electrostatic forces exerted on the cantilever or by intrinsic mechanical stresses generated within the cantilever or on the cantilever surface as a result of the analyte interactions [6]. Examining the degree of cantilever bending eliminates the difficulty of viscous damping for experiments performed in a microfluidic chamber that apply a laminar fluid flow around a cantilever, particularly for large analyte sample volumes, reducing the noise generated by fluid turbulence [7]. Isotropic cantilever materials tend to either decrease the surface area (tensile stress) or increase it (compressive stress) as a result of uniform surface stress (σ_s), as we will discuss later. It is worth mentioning that if a cantilever fails to compensate for surface stress equally, the structure can be permanently bent at the opposite sides of a beam or thin plate.

In the static cantilever model, main readout mechanisms include piezoelectric, optical beam deviation or interferometric deviation measurement, and a variety of amplifiers are employed according to the technique. As an interesting example of the static cantilever model, the readout circuitry employs an array of four cantilevers connected to the amplifiers through an analog multiplexer [8]. Moreover, there is a non-aligned cantilever plane that is not deformed between the locations of tensile and compressive stress. Due to the bending deformation, a force F acts at a distance x , resulting in a bending moment $M = Fx$; hence, using the moment of inertia I , and Young's modulus E , the cantilever's radial curvature can be calculated from equation (8.1) [9];

$$\frac{1}{R} = \frac{d^2z}{dx^2} = \frac{M}{EI} \quad (8.1)$$

In the static mode, receptor target binding at the molecular level can be converted into an electrical signal via optical beam deflection, interferometric, piezoresistive, piezoelectric, capacitive, and electron tunneling readout approaches [10–12]. An analysis of the sensitivities reported to date, including the resonance frequencies at which these sensitivities are measured, has been reported in detail by Stemmer *et al* [13]. Any well-designed detection system can achieve a performance such that the shot noise of the electro-optic system is the lowest possible noise background. The detection system noise level determines the measurement sensitivity. Moreover, microcantilever-based sensing platforms can be used in atomic force-based surface imaging to obtain the surface topography of samples from the sensing tip of an AFM [14].

Bicell or quadrant photodiodes are the most used sensors in optical deflection (OBD) sensor systems. In the OBD method, a beam of light (usually laser light) is focused on the cantilever end, and the light is reflected onto the photodiodes. Deviation in the cantilever causes a change in photocurrents in each cell of a quadrant photodiode. Arithmetic differences of photocurrents between quadrants are proportional to cantilever deviation. Nowadays, using this method, cantilever static deviation can be measured with a high precision, and it is successfully used in the resonating mode at bandwidths up to the MHz scale.

A myriad of detection and sensing applications have been developed for the other biological entities, including DNA, RNA, proteins, metals, antibodies, pesticides,

antigens, cancer biomarkers, and explosives [15–24]. From the virus sensing and detection perspectives, a triangular microcantilever sensor was developed to detect cantilever-based mass and liquid-borne particle sampling with a resonant frequency of 271 kHz, a sensitivity of 0.13 Hz pg^{-1} , and along with the Q (quality) factor of ~ 3000 . The setup achieved a minimum detectable mass of $\sim 0.05 \text{ pg}$, and can be used to monitor liquid-borne viruses (e.g. coronavirus) (figure 8.1) [25].

8.1.2.2 Resonating cantilevers

Resonating cantilevers are designed to vibrate at a particular resonant frequency. The main materials used in their fabrication are a range of single-crystal materials with micron-scale dimensions. Mainly, the vibration signal of cantilevers is converted to electrical signals by optical beam deflection, interferometric and piezoresistive sensing methods, due to higher achievable bandwidths, greater measurement accuracy, and ease-of-interfacing. Resonating cantilevers may be triggered with mechanical or acoustic waves generated by piezoelectric materials, as well as magnetic, electrostatic, or optical stimuli. Frequency shifts in the cantilever can be detected by phase-locked-loop systems. The frequency of the resonating system can be determined as a function of the measurand, which alters the shape or mass of the resonator by chemically sensitive material coupling or by depositing material onto the resonator. The other components are an amplifier, a detection module, and a vibration drive; vibrations are excited, then sensed in the detection module, and fed back to the drive mechanism via the amplifier. Driving

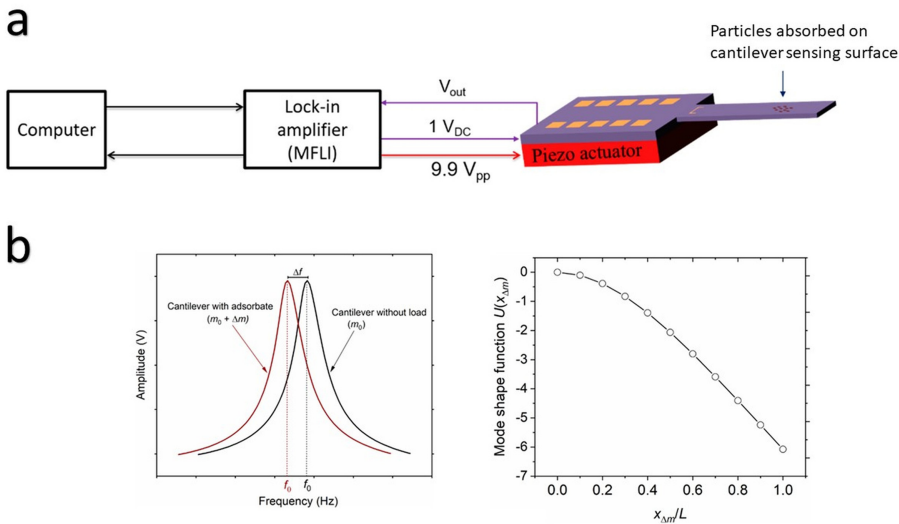


Figure 8.1. (a) A schematic setup for cantilever measurement: a lock-in-amplifier (MFLI) supplies voltage to the in-plane piezo actuator and Wheatstone bridge of a piezoresistive silicon-based cantilever sensor. (b) (i) The frequency shift (Δf) observed in the resonance of a cantilever following exposure to an adsorbate (Δm). (ii) When a uniform beam vibrates under a continuous force at a natural frequency, the deformation data can be shown as the mode shape function, which is obtained at different points along the cantilever. Reproduced from [25], with permission from MDPI. CC BY 4.0.

the efficiency of the vibration excitation and detection mechanisms can be enhanced by modifying the location and the geometry of the electrodes [26, 27].

The resonator design characteristics can be determined using the Q factor (Q). It can be defined using equations (8.2) and (8.3) [27]:

$$Q = 2\pi \left(\frac{E_{\text{stored}}}{E_{\text{lost}}} \right) \quad (8.2)$$

$$Q = \frac{f_0}{\Delta f} \quad (8.3)$$

where E_{stored} is the stored total energy, and E_{lost} is the energy lost per cycle because of damping effects. Moreover, f_0 corresponds to the resonant frequency with the maximum amplitude, and Δf represents the resonance difference of vibrations that are 3 dB lower than the maximum amplitude. The frequency shift measurement accuracy is proportional to the Q factor, and the higher Q factor corresponds to a higher detection limit. On the other hand, the coupling of the resonator results in a reduction in its resonant frequency since exposure to surrounding liquids or gases increases the effective inertia of the resonator [26, 28].

To explain this condition, a myriad of models have been established, including flexural, in-plane, torsional, and extensional oscillations for low-order modes of cantilever beams using two-dimensional analysis [29]. These studies show us that fluid viscosity has a crucial role in the frequency response, especially for two-dimensional flow fields, since the cantilever beams have a high aspect ratio (length/width) [30]. For the 3D forces, fluid compressibility is a crucial parameter affecting the flexural modes for arbitrary mode orders [31].

The sensing abilities of resonating cantilever-based biosensors offer label-free quantitative measurement of biological entities, including viruses, proteins, cells, and nucleic acids using a variety of interfacing electronics and mechanical structures [29, 32]. In particular, silicon-based materials are favored thanks to their low dissipation and high Q factor. Moreover, polymers with high moduli, piezoelectric and magnetoelastic properties have been used as cantilever fabrication materials [33–35]. The fluid cell structure, the inlet and outlet ports are organized in such a way that they are less affected by the noise due to the flow turbulence. Likewise, special immobilization and cleaning processes prevent unwanted behaviors of the system from occurring. In addition, many designs have been studied for single- or multiple layers with either uniform or non-uniform surfaces at appropriate length scales. The surface functionalization on the biosensor surfaces used for biorecognition processes can be accomplished using three steps: (i) surface chemistry preparation, immobilization, and passivation. Subsequently, two biosensing methods can be applied, either continuous measurement of the resonant frequency or measurement of the extremes of the resonant frequencies. For example, an electronic system for a transducer based on a lateral resonating cantilever was developed to predict the dynamic and static behaviors of an electrostatically coupled cantilever-driver structure (CDS) using AC and DC voltages from a driver electrode placed closely

parallel to the cantilever. For different voltage polarizations, the frequency response, the static deflection, and the oscillation frequency response were calculated by accounting for the 3D effects, including the fringing field contribution and the real bending of the cantilever, which are relevant in CDSs with low width/gap ratios [36]. In another example, a mass-sensitive cantilever resonating in an air–liquid environment was designed to detect resonance frequency, surface tension changes, and fluorescent intensity changes in 20, 40, and 80 $\mu\text{g mL}^{-1}$ concentrations of rabbit anti-mouse IgG antibodies on the sensor surface. The resonating properties of the cantilever were characterized as follows: a 50% larger Q factor, a 62% larger vibrating amplitude, and a signal-to-noise ratio 5.7 times higher than a completely submerged cantilever; the surface tension was also observed between 57 and 71 mN m^{-1} with a slit width of 6 μm [37].

To benchmark the analytical performance of the device/sensor, biological entities such as bacteria, viruses, nanoparticles, or small molecules, are measured the most. Sensors with very high absolute mass sensitivity can allow analytes to be recognized and counted individually. As shown in some studies (figure 8.2), doubly clamped very-high-frequency nanomechanical cantilever systems can accurately weigh as little as xenon atoms with a noise floor limit of detection (LOD) of ~ 20 zg and a resolution of 7 zg or 7×10^{-21} g [38]. As label-free biosensors, these systems have been applied to the detection of biological samples, such as viruses, single cells, proteins, and individual strands of DNA [3, 39].

8.1.3 Acoustic wave and piezoelectric micromechanical biosensors

Acoustic waves have been used commercially for over half a century in communications, automotive, and environmental applications. Applying radio frequency or alternating current excitation to electrodes on a piezoelectric material generates acoustic waves that spread across a material surface or perpendicularly form into the bulk medium [40]. The acoustic wave sensor, first reported by Sauerbrey *et al* [41] in 1959, demonstrated the applicability of acoustic waves in biochemical sensing using a bulk thickness shear known as a thickness shear mode (TSM), the mode in a quartz crystal microbalance (QCM), to measure the weight of a thin film by detecting its resonant frequency [42]. Acoustic wave sensors have been adapted for biosensing in

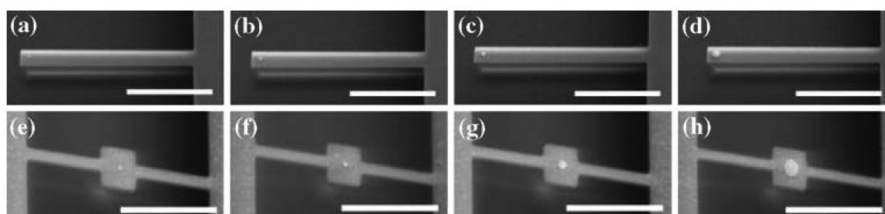


Figure 8.2. A sample of nanomechanical resonators employed in mass sensors with low detection limits. Oblique-angle SEM micrographs of (a)–(d) cantilevers (scale bar = 5 μm) and (e)–(h) bridge oscillators (scale bar = 2 μm). The diameters of the Au pads from left to right are 50, 100, 200, and 400 nm. Reprinted from [230], with the permission of AIP Publishing.

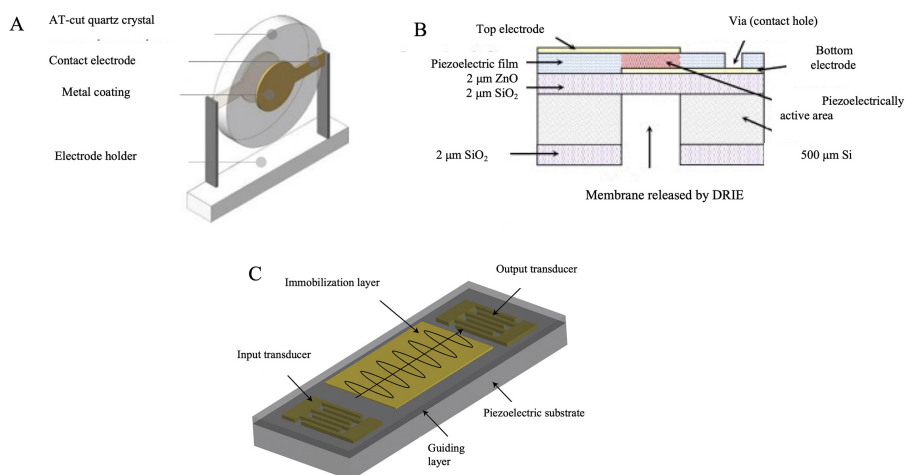


Figure 8.3. A schematic representation of acoustic wave micromechanical sensors. (a) A QCM sensor consisting of an AT-cut quartz crystal coated with two gold electrodes. Reproduced from [231], with permission from MDPI. (b) The schematic cross-sectional view of an FBAR device. Reprinted from [232], Copyright 2012, with permission from Elsevier. (c) A schematic representation of a shear horizontal surface acoustic wave (SH-SAW) device. Reprinted from [233], Copyright 2016, with permission from Elsevier.

recent years due to their high precision, ease of handling, and their ability to process small amounts of biofluids [43]. In addition, acoustic wave-based devices are advantageous because they are less expensive than other sensors without compromising on sensitivity [44, 45]. In acoustic biosensor technologies, acoustic waves pass through a surface formed of a piezoelectric material that has been modified to capture the target biomarker. When binding occurs, the mass of the biomarker changes the properties of the waves, and thus a signal is generated [43]. Using its mass, which is an essential property of any analyte, acoustic/mass-sensitive devices allow analytes to be detected without labels [46].

Acoustic wave sensors are classified according to the waves used: surface or bulk acoustic waves. A bulk wave is described as a wave propagating over a substrate [47, 48]. Two essential devices are reliant on bulk acoustic waves: QCMs and film bulk acoustic resonators (FBARs). The most widely used bulk acoustic wave-based device is the quartz QCM, which has two metallic (usually gold) electrodes separated by a quartz crystal (figure 8.3(a)) [45]. QCM devices use a bulk thickness shear mode that is suitable for liquid and dry environments. These devices commonly have good temperature stability and low bogus bulk wave generation [40]. In QCM biosensors, the crystal surface is frequently covered with a specific receptor that binds to the target analyte. When binding occurs, an increase in the hard crystal mass is observed, which causes a change in the essential resonant frequency of the crystal. This sensor signal is used to accurately determine the mass adsorbed on the crystal surface [46]. The ability of QCM sensors to perform real-time measurements facilitates the possibility of using them in automated continuous monitoring systems. This advantageous feature also makes them applicable for the characterization of affinity-based systems [49]. QCM

biosensors are widely used for the detection of bacteria [50, 51], viruses [52, 53], mycotoxins [49], disease markers [54, 55], and many other analytes, even at low concentrations, due to their simple use, low cost, and sensitivity.

The essential operating principle of the FBAR, another bulk acoustic wave-based device, is technically similar to those of QCM devices. FBAR devices have a smaller base mass than those of QCMs, enabling a much higher resonance frequency and eventually providing more sensitivity. Moreover, the operating resonance frequency of FBAR devices is generally significantly higher than those of QCMs (sub-GHz to 10 GHz) [56]. The working principle of FBAR-based mass sensors is reliant on the mass loading effect. Consequently, as the mass loading on the resonator increases, downward shifts occur in the resonant frequency [57]. Unlike QCMs, FBARs employ piezoelectric film instead of quartz crystal as the resonance unit. Hence, they can be developed at small sizes, allowing higher sensitivity detection [58]. FBAR devices typically have a three-layer structure, as shown in figure 8.3(b), consisting of a lower electrode, a piezoelectric layer, and an upper electrode sputtered onto a silicon substrate. When an alternating current signal is applied to the piezoelectric material, the electrical signal is converted into an acoustic/mechanical wave that spreads lengthwise in FBAR devices [57].

Devices based on surface acoustic waves (SAWs) were first designed in 1965 [59], and these devices were utilizing interdigital transducers to generate SAWs. Subsequently, extensive research into SAW sensors for a wide variety of applications began to increase rapidly [60]. Among acoustic devices, SAW devices stand out due to their small size, extremely high frequency, rapid response, high durability, and integration with various receiver materials. These properties make them highly suitable for the detection of chemical warfare agents [61], volatile organic compounds [62], toxic gases and explosives [63, 64], and biological analytes [65, 66]. These devices are made of piezoelectric materials, on which a periodic comb-shaped interdigital transducer (IDT) pattern is created using photolithography or another process. After an AC voltage is applied to an IDT, an acoustic wave propagates that spreads across the crystal surface [67]. SAW devices are grouped into three categories according to the type of surface wave: horizontal shift/Love wave sensors, Lamb wave (L-SAW) sensors, and Rayleigh wave (R-SAW) sensors [68].

Shear horizontal surface acoustic wave biosensors provide precise and cost-effective real-time detection of many analytes and biomolecules without labels, using horizontally polarized surface shear waves. These biosensors typically comprise a planar electrode formed from a piezoelectric substrate that contains IDTs (figure 8.3(c)). Lithium tantalate, meeting most of the requirements for efficacious horizontally polarized surface shear wave production, is employed as a substrate. These waves are produced by IDTs using an AC voltage at a high frequency from 80 to 400 MHz [69]. The shear horizontal wave generated in these devices has a displacement parallel to the substrate. In-plane propagation provides the minimum damping of waves in environments in which the detection area is liquid. Therefore, shear horizontal surface acoustic wave biosensor devices are more suitable for research aimed at detection in liquid environments [70]. Another type of SH-SAW device that is suitable for SAW biosensors is the Love wave sensor, which is defined as the acoustic resonance of an

accumulated layer in which horizontally polarized surface waves move across a substrate [71]. Love wave-type surface acoustic wave sensors are one of the latest designed devices for the incorporation into lab-on-a-chip systems, and they are especially appropriate for DNA, antibody, and protein analyses. Love waves spread to a close vicinity of a piezoelectric material's surface, which supplies horizontal shear waves if placed above a piezoelectric substrate with a lower shear rate. The amplitude and the speed of the wave mainly depend on changes in the near-surface media [72].

Rayleigh waves are the other type of surface elastic wave of the broad acoustic wave family, and they are similar to mechanical waves on the Earth's surface [73]. Rayleigh waves can be defined as guided waves capable of propagating through a semi-infinite solid medium surface. This wave preserves the majority of its energy, compared to other kinds of body waves such as S-waves or P-waves, and it also decays very slowly at the surface when the half-space is subjected to a harmonic load or displacement [74]. R-SAW sensors are commonly used as gas sensors, in which role they are responsive, yet they are not suitable for real-time detection in liquids due to SAW damping. The high sensitivity of R-SAW sensors occurs because their wave energy is naturally limited to the substrate without the need for a guiding layer [75]. On the other hand, Lamb wave devices support two different spreading approaches: symmetrical and anti-symmetrical, which reflect the symmetry of particle motion around the plate's median [76]. Recently, as a good alternative to the operational limitations of SAWs, Lamb wave-based sensors have been designed to work with acoustic flow, sorting, mixing and particle patterning in a sessile droplet [77]. Generally, a Lamb wave is elliptically polarized with two-particle displacement components, namely shear vertical and longitudinal. According to the wavelength ratio of the specific plate thickness, a Lamb wave has a predominantly in-plane longitudinal component with a near-zero shear vertical component [78].

The piezoelectric effect is the phenomenon whereby electrical charge forms on certain materials due to the application of mechanical stress. This effect is a reversible process, meaning that a volume change can be induced by an electric field [79]. Therefore, piezoelectric materials are perfect for the transducer applications. Piezoelectric materials have recently garnered significant attention for sensor applications. They are composed of various substances, such as organic materials (e.g. polylactic acid (PLLA) [80] and polyvinylidene fluoride (PVDF) [81]), inorganic materials (e.g. lead zirconate titanate (PZT) [82], and aluminium nitride (AlN) [83]), composites, and biomaterials (e.g. cellulose [84], collagen [85], and chitin [86]). Additionally, silicon dioxide (SiO₂) [87] are also preferred for their high mechanical durability. Although most organic polymers are already biocompatible, inorganic materials may require encapsulation to achieve biocompatibility [88].

In addition, the use of piezoelectric material as a mass sensor causes the frequency of the system to change; such a change can be calculated from Sauerbrey's equation (equation (8.4)) [40, 89]:

$$\Delta f = \frac{2\Delta m f_0^2}{A\sqrt{\rho\mu}} \quad (8.4)$$

where Δm is the mass loading, f_0 is oscillation frequency, p is the density of the piezoelectric material, A is the surface area and μ represents the ratio of the mass to the area. Additionally, a coupling factor, k^2 , is utilized to perform the conversion between electrical energy and mechanical energy (equation (8.5)) [40, 90]:

$$k^2 = \frac{e_{31}^2}{c_{11}\epsilon_{33}} \quad (8.5)$$

where c_{11} , e_{31} , and ϵ_{33} represent the electrical field, the elasticity of the material, and absolute permittivity, respectively. The coupling factor value depends on the type of piezoelectric material used, the composition of its guiding layers, and the thicknesses of the layers.

The sensing of biological and chemical markers on a deposited piezoelectric surface is achieved by analyzing changes in the mechanical stress, electrical charge, and applied mass. For instance, piezoresistors are utilized with MEMS to sense low concentrations of markers by measuring the surface stress of the material. Temperature is the main drawback of this method. However, utilizing four piezoresistors as a Wheatstone bridge may overcome this issue [91].

Strain caused by periodic external stress leads to wave propagation inside piezoelectric materials. This longitudinal or transversal wave defines the frequency range of the device [89]. The wave spectrum utilized by piezoelectric biosensors consists of many modes, such as the acoustic plate mode (APM), thickness shear mode (TSM), SAWs, and the flexural plate wave (FPW) mode [40]. For instance, the FPW mode is a vibrating mode that is induced by a substance composed of piezoelectric material and structures resembling cantilevers or membranes. The stiffness coefficient of the cantilever is changed by the binding of molecules to the functionalized surface. This affects the resonance of the sensor, since the bending moment on the cantilever is altered, and markers are detected on the surface [92]. In a study, FPW devices were integrated with self-assembled monolayers (SAMs) followed by the immobilization of immunoglobulin-E (IgE) antibodies for allergen detection [93]. Additionally, piezoelectric materials are new alternatives to conventional semiconductor materials in micromachined ultrasonic transducers (MUTs). These devices are especially promising for medical diagnosis, fingerprint recognition, and implant power supplies [94].

Immunosensors composed of piezoelectric materials detect specific antigen groups and measure their concentrations using immobilized antibodies on their surface along with favorable surface functionalization. As an antigen attaches to the immobilized antibody, the frequency of the system oscillations alters, and biorecognition occurs. Antigens with high molecular weights are preferred since their increased stimulation causes a more significant decrease in the oscillation frequency. However, this effect can also be obtained by introducing nanoparticles covered with antibodies [95].

8.1.4 Surface stress-based biosensors

The surface stress, σ_s , is the magnitude of work per unit area required to stretch a surface (figure 8.4) [96]. Intermolecular forces arising from adsorbate–surface and

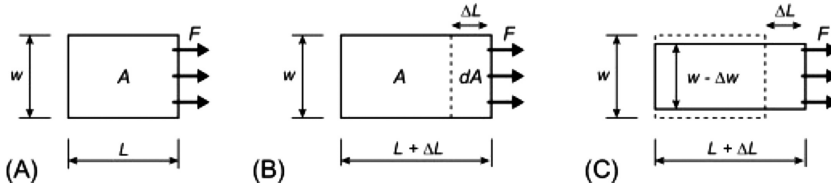


Figure 8.4. Surface stress on a cantilever. (a) A stretching force acting on a surface. (b) Graph of one-dimensional deformation. (c) A design that considers lateral deformation. Reprinted from [96], Copyright 2019, with permission from Elsevier.

adsorbate–adsorbate interactions of small molecules are known to be factors that induce surface stress, which results from the mechanical bending of a solid substrate [97]. The surface stress σ_s and F are given by equations (8.6) and (8.7):

$$\sigma_s = \frac{dW}{dA} = \frac{F}{w} \quad (8.6)$$

$$F = \sigma_s \cdot w \quad (8.7)$$

where w , L , A , and F are the width, length, area, and force, respectively. In this scenario of one-dimensional stretching (see figures 8.4(a) and 8.4(b)), the new area $dA = w \cdot \Delta L$, and the work $dW = F \cdot \Delta L$.

The concept of surface stress has been known since the 1900s, and it was first applied at the end of the 20th century for sensing studies [98–100]. The hypothesis of surface stress is derived from the minuscule repulsive or attractive forces that can be generated on a cantilever surface because of the alterations in its electronic energy states or charge distribution when target–receptor interactions occur [101]. Surface stress applied to a cantilever can be described by Stoney’s formula using different dimensions (force per length) when the film thickness is very small or it obeys an unknown function (8.8) [102]:

$$\sigma_s = \frac{Et^2}{6R} \quad (8.8)$$

where E denotes Young’s modulus, t represents thickness, and R is the radius of curvature resulting from the interactions of molecules with the thin-film cantilever. Furthermore, the deflection, δ of a cantilever system can be explained by the bending obtained from the radius R and the length, L , of the cantilever curvature equation (8.9) [96, 102]:

$$\delta = \frac{3L^2}{Et^2} \sigma_s \quad (8.9)$$

From a chemistry perspective, it can be explained by an atomic bonding at the surface. If bond strengths between surface atoms are more powerful than those between subsurface atoms, tensile surface stress is generated by attractive forces, resulting in a concave surface curvature. On the other hand, if the surface atoms tend

to repel each other, compressive surface stress is induced, resulting in a convex surface curvature [103, 104].

In the early developmental stage of surface stress-based biosensors, scientists made a good analogy between biology, in which the interactions of membrane molecules result in the lateral tension of a lipid bilayer and a cantilever system [105, 106]. This technology includes a relatively new class of biosensor, which encompasses three main features: (i) a transporter of analytes, (ii) a sensitive element that has a selective layer and a cantilever or membrane as a transducer, and (iii) a signal detection system [104]. Such biosensors can be described as microscale and label-free systems that work in the reported range of 1 mJ m^{-2} to 900 mJ m^{-2} [97, 107–110].

The transporter of analytes is geometrically restricted to a small size to meet the requirements for microdomain forces and low energy consumption. Microfluidics is generally employed to mimic conventional analyte handling techniques, which enables the use of facile fabricated, highly integrated systems, allowing the rapid analysis of biological materials [111]. The sensitive element of such sensors highlights the ability of surface stress sensors with selective layers to detect a myriad of reactions, e.g., antigen–antibody binding [112], DNA hybridization [113], SAM formation [114], and phase-transitive reactions [115]. The transducer layer can either be a membrane or a cantilever. This element basically has two main layers: a submaterial layer (such as silicon, aluminum nitride, silicon nitride, polymethylmethacrylate (PMMA) or polydimethylsiloxane (PDMS)) and a contact layer, i.e. a gold layer. For instance, a microcantilever-based aptasensor was developed with thrombin DNA aptamer (TBA15–1) as a biorecognition element using a plasma treatment from the top, and a layer of gold was coated from the bottom side and developed using an SU-8 polymer and MEMS lithography [116]. Compared to its silicon counterparts, the deflection value of the SU-8 polymer-based microcantilever was found to be an order of magnitude greater, due to its small Young's modulus. However, cantilevers are mostly developed stiff substrates with low selectivity and anisotropy, resulting in very limited sensitivity, yet good biocompatibility. Moreover, cantilever geometry is not the best option for sensing in liquid media, and thus, it can severely reduce the signal-to-noise ratio (SNR) because of non-specific adsorption on the backside of the cantilever [117]. Hence, membranes are more convenient than cantilevers for sample isolation and detection. For example, a surface stress-based capacitive biosensor was developed via the structural optimization of a micromembrane consisting of a PDMS submembrane coated with gold that served as a sensitivity layer. The membrane shape, the coverage of gold, and the thermal strain mismatch were optimized using deflection and capacitance finite element analysis (FEA, ANSYS®). The sensor responses to reactions causing deflection for a surface stress change σ_s and the equivalent temperature load ΔT were calculated using equations (8.10) and (8.11), respectively [118]:

$$\frac{1}{R} = 6 \left(\frac{1 - \nu}{Et^2} \right) \sigma_s \quad (8.10)$$

$$\Delta T = \frac{\sigma_s}{E_g \cdot \partial_g \cdot T_g} \quad (8.11)$$

where ν is Poisson's ratio, E_g is Young's modulus, ∂_g is the coefficient of thermal expansion, and T_g is the thickness of gold used. The results demonstrated that the geometry and gold coverage of the membrane affects the deflection rate and the sensitivity of the sensor system.

Signal detection setups can generally be classified into those based on electrical detection and optical detection. The optical systems can be categorized into those based on radioactive [119], fluorescent [120], and fiber interferometry detection [121]. The electrical systems can be further classified into those based on piezoresistive [122] and capacitive detection [123]. In principle, microcantilever approaches depend on easy and rapid optical methods to detect deflections without any internal sensing material that can disturb cell environment. On the other hand, the thermal effect of heat drift constitute a disadvantage that causes an excessive temperature increase in the cantilevers [124]. From an application perspective, a surface stress biosensor utilizing a MEMS-based Fabry–Perot interferometer for label-free sensing of an antigen–antibody interaction was designed on a movable parylene C membrane with 300 nm of Au film. By editing the spring constant and coverage ratio of the Au half-mirrors, the sensitivity and selectivity were modified to detect the amount of deflection. The wavelength selectivity was improved by 6.6 times through integrating these mirrors further to that a reflection peak shift since bovine serum albumin (BSA) antigen–antibody interaction was observed spectroscopically using the fabricated optical interferometer [125]. Compared to optical detection, electrical detection is a relatively low-cost and facile system that can be used with opaque solutions, yet its fabrication process is complex, and the stiffness of the materials used is larger than those of the usual sensing elements, thereby reducing the sensitivity. From an application perspective, a piezoresistive membrane-type nanomechanical cantilever sensor created by boron diffusion onto single-crystal silicon has emerged for the label-free and real-time detection of analyte-induced surface stress. The adsorbate membrane consists of four piezoresistive sensing beams, consisting of a full Wheatstone bridge, which is ~ 43 times more efficient than a standard cantilever (figure 8.5). The relative resistance change ($\Delta R/R$) was evaluated by FEA (COMSOL Multiphysics 3.5a) by applying compressive surface stress (-3.0 N m^{-1}), resulting in downward deflection and tensile stresses in the x (σ_x) and y (σ_y) directions, where π_{44} ($\sim 138.1 \times 10^{-11} \text{ Pa}^{-1}$) is a typical piezoresistance coefficient in the equation as follows (8.12) [126]:

$$\frac{\Delta R}{R} = \frac{1}{2} \pi_{44} (\sigma_x - \sigma_y). \quad (8.12)$$

8.2 Virus detection using micromechanical biosensors

In history, we have faced many clinical conditions and diseases; however, infectious diseases have gained the attention of researchers because they can spread to other individuals in various ways, such as through contact, contaminated air, water or

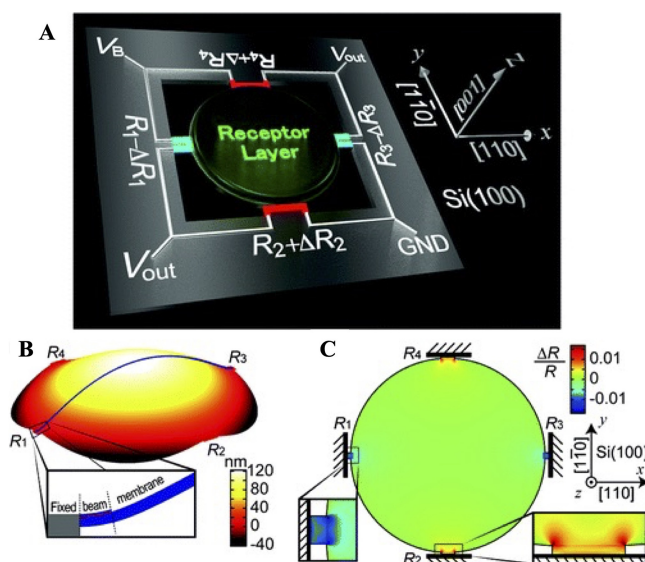


Figure 8.5. (a) A schematic illustration of the membrane-based sensor with p-type piezoresistors on a single crystal of n-type silicon. (b) The deformed shape of the membrane. (c) The distribution of $\Delta R/R$ on the surface of the sensor with current flowing in the x direction. Reprinted with permission from [126]. Copyright 2011 American Chemical Society.

food [127, 128]. Therefore, controlling infectious diseases is crucial to maintaining global health. In addition, socio-economic, environmental, and ecological factors are also strongly influenced by infectious diseases [127]. In the last decade, infection-related mortality and morbidity have increased at an alarming rate. More specifically, the various types of viruses (e.g. HIV, dengue, ebola, Zika, influenza, hepatitis, norovirus, severe acute respiratory syndrome (SARS), and SARS-CoV-2) have a significant impact on multiple aspects in society, science, and technology [129]. Traditional diagnostic procedures are mainly based on enzyme-linked immunosorbent assay (ELISA), nucleic acid detection (e.g. a polymerase chain reaction (PCR)), culturing, or microscopy, which are complex, time-consuming, and expensive, and also they require sophisticated instruments and highly skilled personnel for their proper operations [130]. As a consequence of these facts, simple, rapid, accurate, portable, multiplex, and inexpensive diagnostic platforms are among the primary concerns of research into early detection, which is a crucial clinical step required to initiate effective treatment [128]. Considering the drawbacks of conventional detection techniques, the necessity for a novel detection platform cannot be ignored. Therefore, recent advances in biosensor development offer crucial solutions and provide rapid, cost-effective, and facile platforms. They present notable improvements in sensitivity and multiplexed sensing modalities for point-of-care (POC) settings [130, 131].

8.2.1 Coronavirus

Over the last few decades, three major types of pathogenic coronavirus, i.e. SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, have spread to large territories, resulting in a high rate of morbidity and mortality [132, 133]. SARS-CoV was the first coronavirus that emerged in China in 2002, spreading rapidly to more than 30 countries and leading to about 8000 SARS-CoV cases [134]. Later, in 2012, a new type of coronavirus known as MERS-CoV was discovered in Saudi Arabia. It was found that camels transmitted it to humans, and it spread to over 27 countries, with a total of 858 cases recorded worldwide [135]. The latter, SARS-CoV-2, was detected in China in December 2019. The impact of the COVID-19 pandemic continues all over the world [136]. This virus spreads rapidly, and it causes serious health problems, such as difficulty in breathing, limb pain, fever, and severe coughing [129]. A patient infected with SARS-CoV-2 takes two to seven days to display symptoms, so about half of all patients are asymptomatic. Therefore, it can easily be transmitted from a patient to a healthy person [137]. According to a survey from the World Health Organization (WHO), over 264 million COVID-19 patients have been identified and over 5.2 million people have died up to December 2021.

In order to suppress the spread of the virus, it is essential to monitor and track the COVID-19 status of persons. A wide variety of imaging techniques, including chest computed tomography (CT) scans [138], single-photon emission computed tomography (SPECT), and positron emission tomography (PET) have been utilized for COVID-19 detection. These methods, however, have low sensitivity, and they are bulky and expensive. Moreover, they can only be performed by trained professionals in centralized hospitals [139]. Furthermore, these methods cannot be easily integrated into the POC settings for broad applicability in screening studies [140]. Briefly, current COVID-19 detection methods include ELISA [141], loop-mediated isothermal amplification (LAMP) [142], chemiluminescence [143], CRISPR (clustered regularly interspaced short palindromic repeats) [144], and reverse transcription-polymerase chain reaction (RT-PCR) assay [145]. Antibody-based detection methods, such as ELISA, are not suitable for early diagnosis. Moreover, CRISPR and immunoglobulin G (IgG) can only be detected in the patient's blood after five to ten days. Another common issue in this process is false-positive results caused by the interference of other proteins in human blood [146]. Although RT-PCR is the gold-standard method for SARS-CoV-2 detection, it is laborious, time-consuming, and it requires a trained person to carry out the process, including purification, nucleic acid amplification, and fluorescence detection. Moreover, this method has a high false-negative ratio (FNR), and people who have false-negative results can spread viruses and infect other individuals [147].

On the other hand, biosensors are powerful tools for COVID-19 detection, offering high sensitivity, high accuracy, and fast turnaround. Majorly, biosensors are classified as optical, electrochemical, electrical (FET), and mass-based biosensors [148, 149]. Due to their advantages, piezoelectric biosensors, also known as mass-based mechanical biosensors, have piqued the interest of researchers seeking to

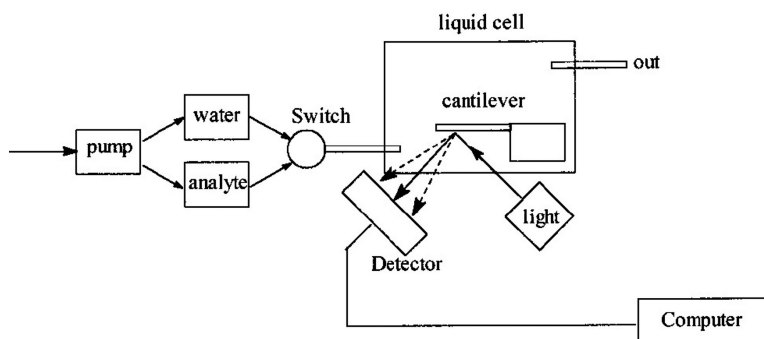


Figure 8.6. The schematic diagram of the instrument used to measure cantilever deflection and demonstrate the glass flow cell. Reprinted with permission from [234]. Copyright 2001 American Chemical Society.

detect COVID-19. In 2004, a QCM piezoelectric immunosensor was developed for SARS-CoV detection from the sputum gas phase. Briefly, horse polyclonal antibodies were immobilized on a crystal surface through protein A in order to achieve an oriented antibody structure. The viruses were specifically adsorbed by the antibodies after an incubation. The mass load on the sensor was changed due to virus adsorption on the crystal surface, leading to frequency variations. The frequency was linearly correlated to the SARS-CoV concentrations, with a dynamic measurement range of $0.4\text{--}6\ \mu\text{g mL}^{-1}$, and an analysis time of less than 2 min. The biosensor exhibited good reproducibility, and the device could be reused 100 times without the loss of activity. In addition, the sensor remained stable for two months while kept over silica gel blue at $4^\circ\text{C} - 6^\circ\text{C}$ [150].

In another study, a microcantilever sensor was utilized for feline coronavirus (FIP) detection. A polyclonal type I FIP anti-viral antibody was immobilized on the cantilever surface. The cantilever bending and its deflection were measured using the optical beam deflection method as the sample approached the unit cell of the sensor (figure 8.6). This research achieved a limit of detection of $0.1\ \mu\text{g mL}^{-1}$ and an assay running time of less than an hour. This work provides an opportunity for the further utilization of microcantilever sensors for the detection of human-associated SARS-CoV [151].

Recently, a MEMS biosensor conjugated with PZT piezoelectric discs was developed for COVID-19 detection. The piezo discs were coated with a sialyl glycopolymer cross-linker polymer receptor to increase the induced surface stress and SL-polyacrylamide (SL-PAA) in order to target the particular protein found on the virus for enhancing device specificity and signal amplification. As the sample was injected, the radial mode resonance frequency shifted due to the surface stress caused by the addition of the sample and the viruses. It was demonstrated that the resonance frequency shift and the virus concentration had a linear correlation. It was also shown that as the piezoelectric disc's thickness decreased, the sensitivity increased. In this study, the SARS-COV-2 concentration was measured in the range of $10^4\text{--}10^8\ \text{virions mL}^{-1}$ [152].

8.2.2 Human immunodeficiency virus

HIV is a virus that attacks the host's immune system and causes acquired immunodeficiency syndrome (AIDS). According to the WHO, 38 million people were living with HIV, 1.7 million new infections occurred and ~1 million people died because of HIV-related disease up to the end of 2019 [153, 154]. These statistics emphasize that HIV infection is a primary public health concern, as it causes high mortality and morbidity. HIV first came to light in the 1980s, when homosexual men were found to have advanced and unexplained immunodeficiency. The origin of the virus is thought to be non-human primates (chimpanzees), and it has spread all over the world over the years [153]. The prevalence of HIV infection in low-income countries (especially in sub-Saharan Africa) is much higher than developed countries. The main reason for this situation is low levels of access to high-throughput systems for diagnosis and treatment. The conventional diagnostic procedures consist of nucleic acid tests (NAT), antigen/antibody tests, and antibody tests using blood, serum or oral fluid samples [153].

In one study, for instance, a microcantilever-based principle was employed to detect HIV envelope glycoprotein (Env) gp120 [155]. Accordingly, the cantilever was functionalized using monoclonal antibodies (mAbs) A32 or T8 for specific binding to gp120, and after HIV was captured, the deflection change was compared to the baseline (figure 8.7(a)–(c)). The developed platform proved that cantilever-based biosensor systems are promising methods for HIV-1 infection detection, offering easy-to-use and portable systems [155]. Another study introduced a platform to detect HIV-2 [156]. This platform utilized a label-free immunosensor approach combined

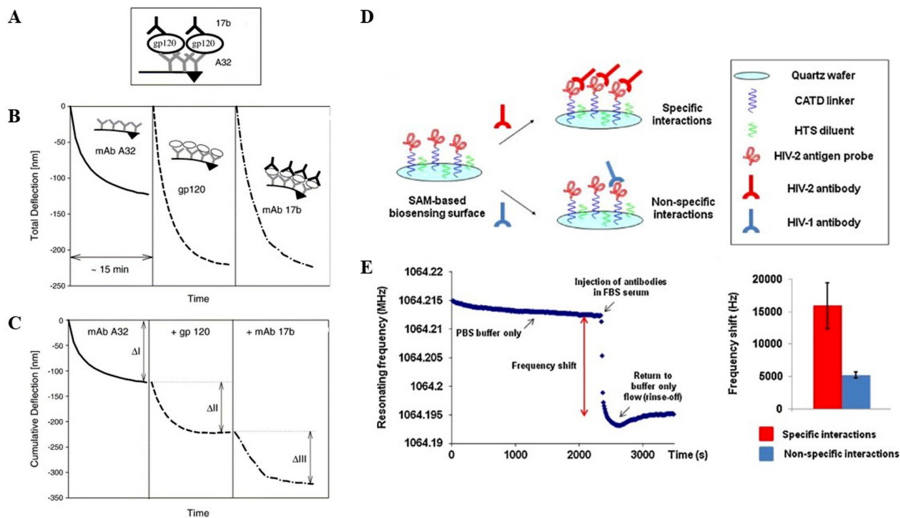


Figure 8.7. (a) A cantilever functionalized with SAMs and antibodies. (b) Total deflection and (c) cumulative deflection changes with respect to time. Reprinted from [155], Copyright 2006, with permission from Elsevier. (d) The illustration of QCM surface functionalization. (e) The frequency change was observed when antibodies were injected into the QCM sensor. Reprinted from [156], Copyright 2011, with permission from Elsevier.

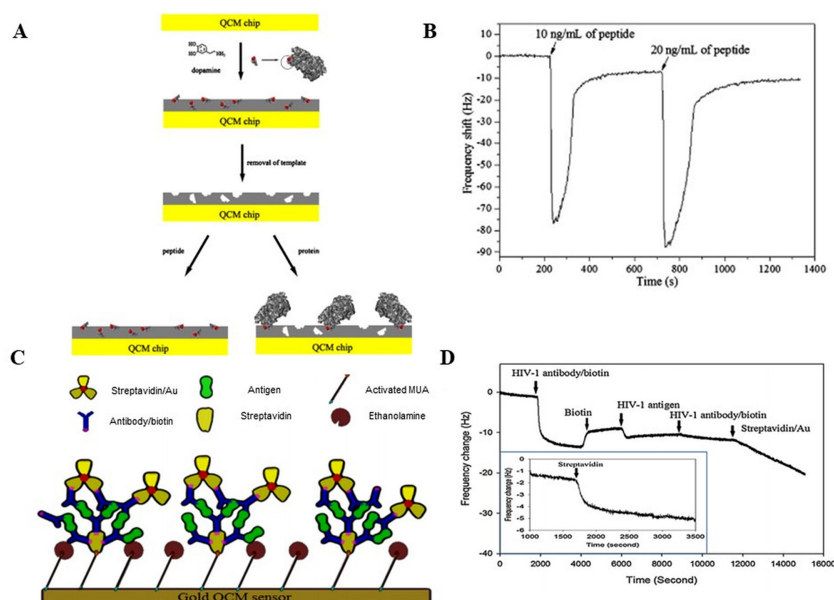


Figure 8.8. (a) The schematic representation of epitope imprinting. (b) The frequency change was obtained when the template was applied in two different concentrations (10 ng mL^{-1} and 20 ng mL^{-1}) and returned to the baseline. Reprinted from [157], Copyright 2012, with permission from Elsevier (c) A schematic representation of surface functionalization with MUA, streptavidin, biotin–streptavidin conjugation, antibody–antigen interactions, and streptavidin–Au binding. (d) The frequency change was obtained due to the applied ligands. Reprinted from [158], Copyright 2016, with permission from Elsevier.

with an electromagnetic piezoelectric acoustic sensor (EMPAS). Herein, a quartz wafer surface was functionalized with a self-assembled monolayer and HIV-2 immunodominant epitopes (see figure 8.7(d)). Specificity and selectivity analyses were performed using HIV-2 or HIV-1 monoclonal antibodies [156]. On the other hand, QCMs are among the most popular micromechanical biosensor designs, since a variety of approaches can be employed. In contrast to the classical approach (e.g. antibody or aptamer functionalization), one of the studies employed molecularly imprinted polymer (MIP)-coated surfaces to create specific grooves for accommodating target molecules [157]. In this study, MIPs were fabricated via epitope imprinting techniques using the HIV-1 gp41 protein template (figure 8.8(a)). Epitope-imprinting polydopamine film not only offers template recognition, but also provides the recognition of the corresponding protein. The use of polydopamine was another novelty of this study, since its use in epitope imprinting has not been common compared to that of dopamine, which is often employed in MIPs due to its high stability and biocompatibility. In addition, facile protocols, high stability, and sensitivity are benefits of these platforms [157]. Another QCM technique was adapted to develop a diagnostic tool for HIV-1 detection [158]. This technique was combined with gold nanoparticles (AuNPs) to enhance the signals that detect the window of the HIV infection period (figure 8.8(c)). Briefly, AuNPs were synthesized by reduction,

and streptavidin was then decorated on their surfaces. At the surface chemistry steps, the sensor surface was first treated with 11-mercaptopundecanoic acid (MUA) and streptavidin. Next, biotinylated polyclonal antibodies, which are capable of capturing HIV-1 p24 antigen, were immobilized. This platform is a promising tool for HIV-1 detection from serum samples, and it provides the detection of antigens, as low as 1 ng mL^{-1} [158]. In addition, a piezoelectric biosensor was developed to detect HIV-1 and HIV-2 from human serum. The surface was functionalized with antibodies against HIV-1 and HIV-2. The LOD was found to be 12 tissue culture infective doses (TCID₅₀s) for HIV-1 (approximately 3,000 viral particles) and 87 TCID₅₀s for HIV-2 in buffers. Considering the nucleic acid techniques, this platform offers some advantages in terms of accuracy, the precision of measurement, and operation [159]. In another example, a microcantilever sensor was designed to capture p24 antigen in order to determine HIV infection. The dynamics of the beam were simulated by the COMSOL Multiphysics software to model the stress and displacement. Elastic silicon-based (SiO_2 , Si, and Si_3N_4) materials were used as wafers, since Young's modulus and Poisson's ratio play critical roles in the deflection rates of different geometries. The results showed that SiO_2 and a V-shaped design were the optimum parameters, due to larger amounts of deflection [160].

8.2.3 Hepatitis

Hepatitis, i.e. the inflammation of liver, holds serious health issues, leading to cirrhosis and liver cancer. This inflammation is majorly caused by various hepatitis viruses, including hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D (HDV), hepatitis E (HEV), and hepatitis G (HGV). Briefly, HAV infects an estimated 1.4 million people worldwide per year [161]. It has been estimated that two billion people worldwide have been infected with HBV. Moreover, 360 million people are chronically infected by HBV, and 600 000 die each year from HBV-related liver disease [162]. Currently, approximately 170 million people worldwide are affected by HCV, and about 3–4 million people are infected globally by the HCV virus each year [163–165]. Hepatitis viruses can be classified according to their severity, chronicity, genomic types, antigenic patterns, and transmission mode [166]. Considering the vast number of people worldwide who have hepatitis and the scarcity of cures for all forms of hepatitis, early detection of hepatitis viruses in various bodily fluids is crucial for controlling and limiting the spread of these viruses, as well as reducing liver-related disease, cancer, and mortality rates.

Several attempts have been made over the last two decades to detect specific biomarkers located on the surface of the hepatitis viruses or to capture antibodies produced against hepatitis viruses. Immunoassay is one of the most frequently employed serological methods for detecting hepatitis viruses. It focuses on the interactions between antibodies and antigens (biomarkers) [167]. This method can be expanded to ELISA [168], RIA (radio-immunoassay) [169], immuno-chromatographic assay (ICA) [170], immuno-chemiluminescence [171], and lateral flow immunoassay (LFA) [172]. Generally, in the immunoassay method, an antigen is

captured by an appropriate antibody and the response can be qualitatively or quantitatively measured. This method's key advantages are low reagent consumption, high sensitivity and stability, low LOD, and ease-of-use. However, the immunoassay approach is expensive and time-consuming, rendering it is unsuitable for clinical applications because the approach needs to be faster and cheaper considering its expansion to the POC and resource-scarce settings [173]. Basically, LFA uses a sample pad, a conjugate pad, an analytical membrane, and a wicking pad. Once the sample is introduced, it passes through the LFA device by capillary force, and later on, reaches the detection site where antigens or antibodies in the sample interact with immobilized antibody antigens on the LFA platform, resulting in a colorful signal that can be distinguishable by a naked-eye [174]. While LFA has higher specificity, sensitivity, and a lower processing time than other immunoassay methods, making it more suitable for clinical applications.

The molecular detection technique is another impactful method used for various types of hepatitis virus detection; in particular, HBV and HCV have been detected with high accuracy as shown in the literature [175]. Molecular approaches include real-time PCR [176], NAT [177], and RT-PCR. In summary, the PCR method relies on the amplification of nucleic acids, mainly RNA and DNA. Accordingly, several DNA target fragments are amplified in a single tube after a large amount of the desired nucleic acid is synthesized in a short period of time. The primary application of NAT methods is to amplify and detect RNA. Molecular detection-based methods have higher sensitivity, specificity, and a wider dynamic range than serological methods. They can also be beneficial when serological techniques fail to respond appropriately [178]. In particular, HBV DNA can be identified using the PCR method even if all serological biomarkers are negative [179]. However, molecular detection methods necessitate complex instruments and post-processing, and they are incapable of demonstrating virus viability [175].

A wide variety of biosensors including electrochemical [180], optical [181], and mass-based (mechanical) sensors [129] have been utilized in the literature for virus detection. Piezoelectric biosensors are prevalent among researchers because of their advantages, which include high sensitivity and specificity, low cost, real-time analysis, and label-free detection [182–184]. A QCM biosensor has been developed for HBV DNA detection [185]. In this research, an HBV nucleic acid probe was immobilized on a gold electrode surface using polyethyleneimine adhesion, the polyethyleneimine glutaraldehyde (PEI-Glu) crosslinking method, and physical adsorption. The results showed that as compared to adsorption immobilization, the use of polyethyleneimine adhesion and glutaraldehyde crosslinking leads to the improvements in reproducibility and stability. Furthermore, HBV DNA concentration was able to be measured in the range of 0.02–0.14 $\mu\text{g mL}^{-1}$, and the biosensors provided reusability, so that the dynamic range was not affected after five time-uses of the regeneration procedure. In another study, peptide nucleic acid (PNA) rather than DNA was employed for real-time monitoring of HBV DNA-PNA hybridization [186]. The PNA probe was immobilized on a gold surface through biotin-avidin binding, which replaced the traditional DNA probe for the direct detection of HBV genomic DNA without prior amplification by PCR. The

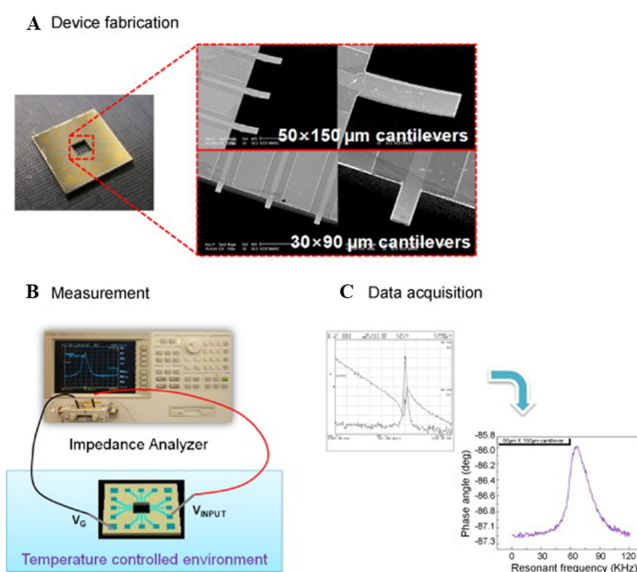


Figure 8.9. Dynamic microcantilever and resonant frequency apparatus measurements. (a) A device composed of twelve microcantilevers and SEM images of two different types of the microcantilever. (b) A schematic representation of an impedance analyzer to determine the resonant frequency. (c) The resonant frequency data was extracted from an impedance signal. Reprinted from [191], Copyright 2009, with permission from Elsevier.

device was relatively fast, and the hybridization assay was completed in 50 min. In addition, the LOD and the specificity were 8.6 pg L^{-1} and 94.44%, respectively. The results were also correlated with those of the gold-standard real-time PCR method, indicating that the fabricated biosensor would be used in clinical applications.

The microcantilever is a mechanical sensor whose material and structure have been greatly improved by developments in the fields of micro- and nanofabrication [187]. Microcantilever sensors have several advantages, including high sensitivity, low-cost, simple operation, and label-free detection, so they can be used as biosensors for DNA hybridization [188], immunoassays [189], and virus detection [190]. For HBV DNA detection, a dynamic microcantilever biosensor enhanced with silica nanoparticles was designed (figure 8.9) [191]. For the DNA target, a 243-mer nucleotide of the HBV DNA precore–core region was used. The detection probe was conjugated with silica nanoparticles, while the capture probe was attached to the microcantilever surface. The detection level of 243-mer HBV target DNA, which was in the picomolar range without the use of nanoparticles, was significantly enhanced by the use of nanoparticles and reached the femtomolar range using the signal amplification strategy. Also, the HBV target DNA concentration provided a linear correlation with the resonant frequency shift. The LOD was 2.3 fM, pointing out that microcantilever biosensors are sensitive and reliable tools for DNA detection.

A Love-wave-mode SAW immunosensor was developed for HBV antibody (HBsAb) detection in whole blood samples. In this design, SiO_2 layers were used

as waveguide layers not only to protect the electrode from liquid media, and also to capture acoustic energy near the surface. To achieve a high sensitivity, the guiding layer thickness was thoroughly investigated. The HBV surface antigen (HBsAg) was immobilized on the sensing element to capture HBsAb while the resonance frequency shift was monitored. A reference SAW device was coated with BSA to eliminate the non-specific binding of HBsAb on the surface, minimizing the impact of other potential factors that could affect the mass alterations. The results demonstrated that the sensor was HBsAb specific and there was a linear correlation between the frequency change and the antibody concentration. The biosensor sensitivity was $0.74 \text{ Hz } (\mu\text{L}^{-1})$, and the LOD was less than $10 \text{ pg } (\mu\text{L}^{-1})$ [192]. In a recent study, HBsAg was successfully detected by a PZT integrated assay [193]. Furthermore, various analytes (HBcAg and AFP) were also studied and a dynamic range of measurement concentrations was obtained by the use of multiple reaction sites in the same assay.

8.2.4 Ebola

The ebola virus disease (EVD), originating from a contagious zoonotic infection, was first identified in 1976. The genus *Ebolavirus*, consisting of negative-strand RNA viruses in the *Filoviridae* family, causes hemorrhagic fever that is also transmitted between humans through bodily fluid contact, with an incubation period in the range of 1–21 days [194, 195]. It invades most major organs and causes pathogenic multisystem failure in humans [196]. Five species are known to be responsible for epidemics: Forest ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus, Reston ebolavirus, and Zaire ebolavirus [195]. EVD is thought to have first caused an infection by direct contact with live or dead animals [197]. The initial symptoms of Ebola are not explicit and they also overlap with other fever-related illnesses; thus, the quick identification of potential cases is crucial to prevent a public health risk holding a mortality rate of 30%–90% [198, 199]. The endemic regions are mainly Central and West Africa, where the availability of medical equipment and trained staff is limited. The current diagnostic methods used for the detection of Ebola viruses could be grouped into three basic categories: (i) serologic tests for the detection of host antibodies IgG [200] or IgM [201], (ii) antigen tests used for the sensing of viral proteins [202] and RT-PCR [203], (iii) molecular (genomic) tests used for the detection of RNA sequences. In particular, sophisticated POC devices could hold the potential to halt future pandemics [204].

From a mechanical biosensor perspective, a reduced graphene oxide (rGO)-based FET platform was used as a biosensor for the real-time detection of the EVD antigen. To improve the semiconducting characteristics of rGO, an AuNP electro-sprayed graphene oxide sheet was used as a conducting channel on the electrodes, and anti-Ebola probes were immobilized to interact with EVD antigens. Part of the rGO sheet passivated by an Al_2O_3 film was created to bridge the gaps between the source and drain electrodes. A fixed drain–source voltage (V_{ds}) was applied between the source and drain electrodes to provide a stable signal. Zaire ebolavirus glycoprotein (EGP) in $0.01 \times$ phosphate-buffered saline (PBS), human serum, and

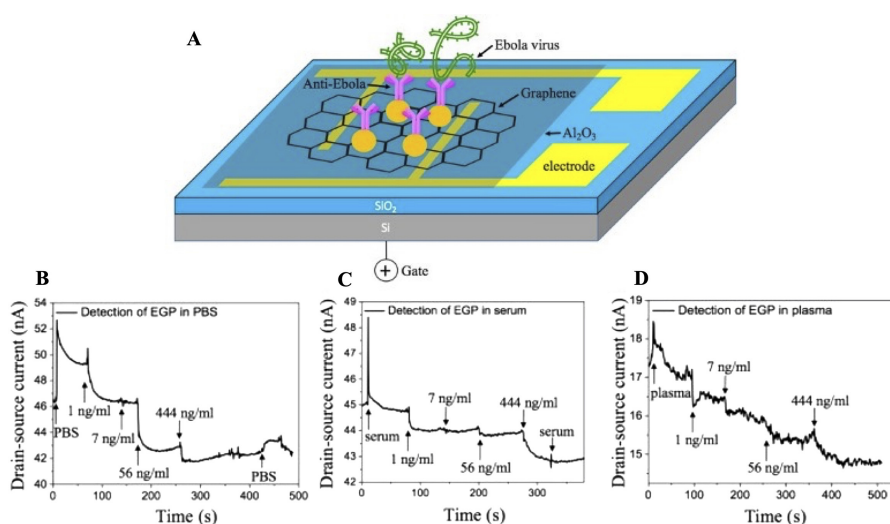


Figure 8.10. (a) A schematic of rGO-based FET biosensor. Dynamic response data of the FET biosensor was obtained from (b) EGP in $0.01 \times$ PBS, (c) EGP in $0.01 \times$ human serum, and (d) EGP in $0.01 \times$ human plasma. Reprinted by permission from Springer (GmbH): Springer Nature, Scientific Reports [205], Copyright (2017).

human plasma solutions were sensed with the LOD of 1 ng mL^{-1} , and the sensitivity increased from 6.5% to 14% (see figure 8.10) [205]. In addition, an SAW sensor and a label-free pathogen-sensing POC device were designed for quick detection of ebola antigens without the necessity for any sample preparation, preparation protocols, or additional reagents. The sensor chips were developed using lithium tantalate (LiTaO_3) wafers and a piezoelectric substrate containing IDTs. When an 80–400 MHz alternating voltage was applied to the IDTs, the polarized surface shear waves were propagated horizontally in the piezoelectric substrate. The interactions between the antibody and the viruses emitted an acoustic wave, leading to a frequency alteration that was observed as a log–linear sensor response with the LOD of $1.9 \times 10^4 \text{ PFU mL}^{-1}$ [206].

8.2.5 Zika virus

The Zika virus, an arthropod-borne virus (arbovirus), is a member of the *Flaviviridae* family and it is transmitted through infected mosquito bites, similarly to other arboviruses, such as dengue and chikungunya. In addition to direct infection from mosquitoes, infected hosts can also transmit viruses to the fetus during pregnancy, sexual contact, the transfusion of blood and blood products, and organ transplantation [207]. The symptoms are not specific to Zika infection, and this generally leads to misdiagnosis [207]. The common symptoms are known to be a macular or papular rash, fever, arthritis or arthralgia, non-purulent conjunctivitis, myalgia, headache, retro-orbital pain, edema, and vomiting. More rarely, hematospemia, transient dull and metallic hearing, swelling of the hands and ankles, and

subcutaneous bleeding may occur [208]. A significant number of patients have mild symptoms, but asymptomatic cases are also known [209].

Diagnosing Zika infection is conventionally carried out using viral nucleic acid (through RT-PCR), IgM antibodies (such as through MAC-ELISA), or a plaque reduction neutralization test (PNT). A definitive diagnosis can be performed using viral nucleic acid from serum; RT-PCR provides conclusive results after one week from the onset of clinical illness [208]. Compared to these methods, a gold-standard diagnosis platform is still a primary necessity. In particular, the massive risk of the cross-reactivity of antibodies for flaviviruses hinders the development of antigenic detection platforms and the interpretation of serologic test results [208, 209]. Moreover, these tests are expensive, hard to conduct, time-consuming, and risky due to the handling of intact viruses. In addition to these limitations, standard reagents are required, which are not easily accessible [208]. Given these facts, the requirement for an accurate, easy-to-use, and cost-efficient detection platform cannot be ignored.

Considering the novel platform requirement, a wide variety of biosensor systems for the diagnosis of Zika infection have been sought by researchers. Micromechanical biosensors are one of the most applied approaches for Zika detection. A recent study, for instance, introduced a QCM biosensor functionalized with aptamers to capture the Zika virus through capsid proteins [210]. The study exhibited three different microbalances, namely a 5 MHz commercial AT-cut (QSense) quartz microbalance, a 10 MHz AT-cut quartz sensor, and a 433 MHz lithium niobate (LiNbO_3) sensor. The third microbalance sensor utilized the SAW principle. A comparison was performed between commercially available QCMs and LiNbO_3 . The sensitivity of the LiNbO_3 sensor was determined to be 370 Hz ng^{-1} with the LOD value of 2.7 pg; the sensitivity of the typical commercial 5 MHz QCM was 1 Hz ng^{-1} and its LOD was 1 ng. The introduced system is promising not only for Zika detection, and it would be adapted to detect other virus types [210].

Microfabricated cantilever beams have been employed as biosensors, utilizing the residual stresses upon to mass accumulation on the surface. A recent study demonstrated cantilever beams and aptamers to detect the Zika virus [211]. The surfaces of the microfabricated silicon nitride cantilever beams were coated with gold disks and triangles to mediate the quantification of residual stresses formed when the Zika viruses were bound to the immobilized aptamers. The tensile residual stresses generated were approximately $\sim 53 \text{ MPa}$. The stress bent the nitride beams and led to a change in their reflective color and apparent length. The sensitivity of the developed biosensor was observed to be 2 kHz ng^{-1} [211].

8.2.6 Norovirus

The noroviruses are members of the genus *Norovirus* within the family *Caliciviridae*, and they are single-stranded positive-sense RNA viruses. Noroviruses are human enteric pathogens transmitted by food contaminated through feces. Vomiting and person-to-person contact are responsible for at least 50% of all gastroenteritis outbreaks worldwide [212, 213]. Norovirus was first identified in Norwalk, Ohio, in

1968 [214]. The kinetics of norovirus outbreaks seem to be connected with complex interaction variables, including environmental and genetic factors [215]. Due to its enhanced ability to survive in the environment, a small inoculum is required to produce infection (<100 viral particles), for prolonged viral shedding [216]. Electron microscopy was first used for determining viruses, but it is a time-consuming method that cannot be applied to the POC settings [214]. Molecular cloning, enabling capsid protein expression and a comparison of the sequences of caliciviruses has revealed four species: lagovirus, vesivirus, norovirus, and sapovirus [217]. Subsequently, the virus was detected either by electron microscopes or immunologic assay methods, such as ELISA and PCR [218]. These systems lack specificity and sensitivity. Besides, it is critical to detect norovirus quickly due to the shedding rate of 10^5 – 10^9 noroviruses per gram of feces for 8–60 days [219]. Here, micromechanical biosensor systems could be a breakthrough in addressing such problems mentioned. For instance, a silica microbead-based microfiltration zone and a graphene and gold nanoparticle (Grp-AuNP) composite integrated electrochemical ferrocene tagged aptasensor on a disposable microfluidic platform were developed to detect norovirus [220]. The Grp-AuNP composite provided a durable substrate for aptamer immobilization and microbeads, which filtered out larger particles or solids from the solution. The interactions between the noroviruses and the aptamer led to a decrease in the electrochemical signal originating from the ferrocene. The aptasensor was easily able to distinguish between the target norovirus and other interfering molecules, along with a detection limit of 100 pM and a detection range between 100 pM to 3.5 nM. In another example, a graphene-based FET biosensor composed of a set of antibody-based affinity reagents was used to catch norovirus on a flexible Kapton substrate manufactured by 3D inkjet printing with a sheet resistance as low as 110 Q sq^{-1} [221]. The change in the S12 (i.e. the voltage gain from source to drain) signal intensity at a frequency of 10 GHz provided a linear dose–response relationship from 0.07 to 3.70 dB when the concentration of norovirus VLP protein increased from 0.1 to $100 \mu\text{g mL}^{-1}$.

8.2.7 Influenza

Influenza, which belongs to the *Orthomyxoviridae* family, is an acute respiratory disease caused by different influenza viruses. It covers diverse subtypes of the virus, including influenza A, B, and C, differentiated by considering the antigenic differences in their nuclear and matrix proteins [222, 223]. Influenza type C causes relatively milder infections and does not create epidemics, compared to influenza types A and B, which cause respiratory illness epidemics, leading to severe impacts on public health through increased rates of hospitalization and mortality [223].

The genome of the influenza viruses is negative-strand RNA that contains eight genes coding for ten proteins, including the surface proteins hemagglutinin (HA) and neuraminidase (NA) [223]. In addition to these three subtypes, the influenza A virus is categorized according to its hemagglutinin, which is divided into 16 serological subtypes (from H1 to H16), and its neuraminidase (NA), which is divided into 9 serological subtypes (from N1 to N9). Regarding their

combinations, approximately 144 possible A/HN subtypes are known; however, not all of them cause diseases in humans. Some of these subtypes have caused pandemic outbreaks, namely A/H1N1 (Spanish flu, 1918), A/H2N2 (Asian flu, 1957), A/H3N2 (Hong Kong flu, 1968), and A/H1N1 again (Swine flu, 1976; Russian flu, 1977) in the last century [224]. In 2009, A/H1N1 caused another pandemic that led to enormous concerns for public health due to the more globalized nature of the world and the pork and poultry production industries [223, 224]. The transmission of these viruses between individuals commonly occurs through large virus-containing droplets and the exchange of aerosols; more rarely, it occurs via contaminated fomites or by direct contact [223].

Several micromechanical platforms have been described in the literature that detect influenza infections aiming to provide low-cost, simplicity, high sensitivity, and rapidity, which would enable the control of transmission and treatment. One of the studies introduced a QCM-based immunosensor to detect influenza types A and B [225]. Here, the QCM sensor employed a frequency or dissipation change due to captured mass. In this manner, antibodies were conjugated with AuNPs to increase the mass sensitivity, and, accordingly, the sensitivity increased tenfold. The LOD value was found to be 1×10^3 PFU mL⁻¹. The sensitivity and specificity of this platform were compared with those of the RT-PCR, shell vial, cell culture, and ELISA methods through a small cohort with 67 clinical samples [225]. Another study also utilized a QCM immunosensor to detect the H5N1 virus, holding an enormous pandemic risk [226]. The study revealed the development of a QCM immunosensor with magnetic nanobeads to enhance the binding reaction between bioreceptor and analytes. Anti-H5N1 polyclonal antibodies were immobilized on a gold-coated QCM surface, which was functionalized with SAMs of 16-mercaptohexadecanoic acid (MHDA) (figure 8.11(a)). When the antibodies captured viruses, a frequency change associated with the concentration occurred (figure 8.11(b)). The developed platform was able to detect the H5N1 virus at a titer of more than 0.0128 HA units within 2 h. The specificity and selectivity of this platform were controlled in the presence of avian influenza subtypes, such as H3N2, H2N2, and H4N8. The sample source was chicken trachea. Consequently, the QCM immunosensor demonstrated huge potential for avian influenza (AI) virus H5N1 detection in clinical, agricultural, environmental, and food samples [226].

Another QCM biosensor was developed to detect avian influenza virus (AIV) H5N1 by combining ssDNA with crosslinked polymeric hydrogel [227]. For the manner of biorecognition, aptamers with high affinity and specificity for AIV H5N1 surface proteins were selected and they formed a hydrogel with the ssDNA. The sensor surface was functionalized with SAMs; the aptamer hydrogel was then immobilized. When the virus was captured by the aptamer, the hydrogel swelled immediately due to the breaking of the bond between the aptamer and the ssDNA; the swelling mediated a frequency shift. The LOD value was determined to be 0.0128 HAUs (HA units) when the aptamer:hydrogel ratio was 1:1. The time required for detection was 30 min, including sampling. This platform offered rapid, sensitive, and specific detection for H5N1 viruses [227].

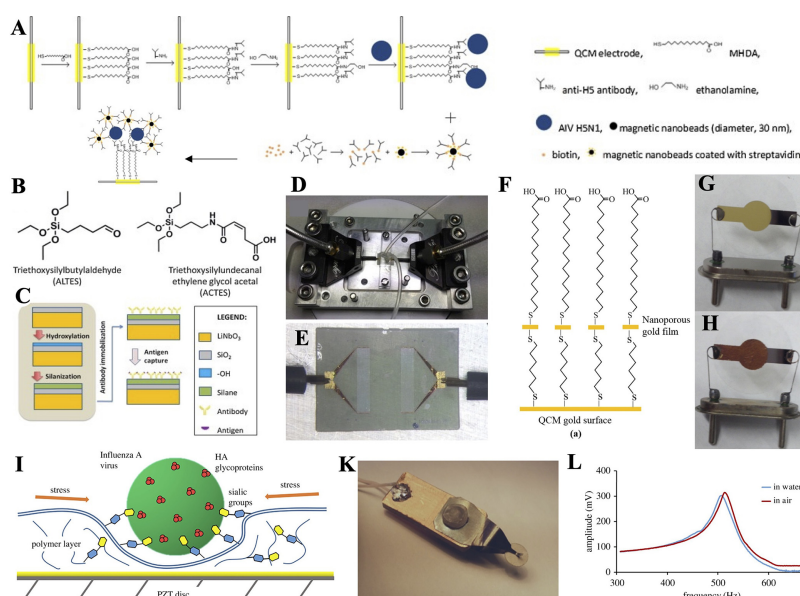


Figure 8.11. (a) A schematic representation of surface functionalization, detection, and amplification. Reprinted from [226], Copyright 2011, with permission from Elsevier. (b) The chemical structure of triethoxysilylbutylaldehyde (ALTES) and triethoxysilylundecanal ethylene glycol acetal (ACTES). (c) The sensor was functionalized via hydroxylation and silanization using *n*-hydroxysuccinimide (NHS) and ACTES; antibodies were then immobilized. (d), (e) The photograph of an SAW sensor. Reprinted from [228], Copyright 2015, with permission from Elsevier. (f) Nanoporous gold film was functionalized on a gold electrode surface to form a nanowell-based electrode. (g), (h) The photographs of a QCM electrode before (g) and after (h) nanoporous gold film was modified. Reprinted from [52], Copyright 2017, with permission from Elsevier. (i) Lateral stress inside a polymer receptor layer with sialyl oligosaccharide groups increased after the binding of virus particles. (k) A photograph of disc and disc holder. (l) The oscillation data of the piezo disc both in the air (red) and water (blue). Reproduced from [229]. CC BY 4.0.

SAW sensors are another class of micromechanical sensors. A recent study introduced an SAW biosensor, which is capable of detecting the influenza A virus using a Love wave modality [228]. The sensor was fabricated using SiO_2 -coated piezoelectric LiNbO_3 wafers. The sensor surface was functionalized with triethoxysilylbutylaldehyde (ALTES) and triethoxysilylundecanal ethylene glycol acetal (ACTES), and influenza A HA antibodies were immobilized on the $\text{SiO}_2/\text{LiNbO}_3$ SAW surface. The LOD value was observed as 1 ng mL^{-1} on the surfaces functionalized with ALTES [228].

Another QCM-based biosensor was introduced to detect H5N1 AIV. In contrast to the other developed platforms, this biosensor was developed using a nanowell (figure 8.11(g) and (h)) structure and an aptamer [52]. The nanowell production employed the metallic corrosion method on a gold film with a $\sim 20 \text{ nm}$ pore size. The nanowell electrode was obtained by nanoporous gold film immobilization with a self-assembled monolayer. The ssDNA aptamers were conjugated with NH_2 in 5-terminal and immobilized on the surface via covalent bonding to construct the

QCM aptasensor. When the virus was introduced to the sensor, AIV H5N1 was bound to the immobilized aptamers; therefore, a resonant frequency changed proportional to the virus concentrations. This platform offers label-free detection of H5N1 infection within 10 min. The detection range of the sensor was determined to be 2^{-4} to 2^4 HAU $50 \mu\text{L}^{-1}$ and the LOD value was 2^{-4} HAU $50 \mu\text{L}^{-1}$. The specificity was controlled by applying other subtypes of AIV (e.g., H1N1, H2N2, H7N2, and H5N3). The study also included validation of the sensor using chicken tracheal swab samples. This platform could also be modified and adapted for diverse types of infectious agents [52].

The most recent study introduced a rapid and label-free piezoelectric biosensor to detect influenza A virus by utilizing a PZT piezoelectric disc's first radial mode oscillations. Briefly, PZT was fabricated from a piezoelectric membrane with a radius of 2 mm and a thickness of 100 μm (figure 8.11(k)) [229]. The sensor surface was functionalized with a synthetic sialyl glycopolymer receptor layer, which has an affinity for hemagglutinin proteins (figure 8.11(l)). When viruses were bound to the polymer layer, the disc's radial oscillation mode frequency was increased. The LOD value was determined to be 10^5 virus particles per milliliter. This platform offers a long lifetime, high sensitivity, selectivity, and easy applicability for home settings [229].

8.3 Conclusions and future perspectives

Viral diseases cause severe health problems for people all over the world. As a result, there is a strong demand for highly sensitive and reliable virus detection methods. Virus detection methods, including immunoassay, molecular detection, and biosensors, have been broadly implemented at multiple settings. A wide range of immunoassay methods, such as ELISA, RIA, ICA, immuno-chemiluminescence, and LFA have been utilized for virus detection. Although they have demonstrated several benefits, their limitations, such as their high-cost and time-consuming nature, have hindered their clinical utility in the early diagnosis of diseases. Molecular detection techniques, such as real-time PCR, nucleic acid tests, and reverse transcription-PCR have many advantages over immunoassay approaches, including higher sensitivity, specificity, and a wider dynamic range. However, these approaches necessitate the use of complicated instruments and pre-processing protocols. Biosensors, on the other hand, are appealing devices for early-stage virus detection, which is a critical step in controlling virus spread and preventing disease. Biosensors offer high sensitivity and specificity, low-cost, real-time analysis, and label-free detection, making them suitable for clinical applications. In particular, this chapter thoroughly investigated various micromechanical sensors, including piezoelectric, surface acoustic wave, and microcantilever biosensors with low detection limits and rapid responses. Furthermore, the most recent developments in micromechanical biosensors for virus detection were extensively reviewed.

Currently, conventional virus detection systems are expensive and only available in centralized clinics and hospitals. Therefore, there is a need to develop a portable POC diagnostic system that can be used by individuals without intensive training. Considering their reliability and rapid response, POC devices are emerging

tools for early-stage viral disease diagnosis. Due to micro- and nanotechnological advancements, POC micromechanical biosensors could be an appropriate candidate for virus detection. Micromechanical biosensors conjugated with surface functionalized nanomaterials can be utilized to improve signal amplification, specificity, bioreceptor immobilization, and sensitivity. The signals could be recorded by an individual's smartphone or any wireless devices that transmit the data to clinical health centers and hospitals, so that trained professionals could monitor individuals' health status remotely. Despite the advances in technology and science in the 21st century, the COVID-19 pandemic has demonstrated that virus infection can lead to high morbidity and mortality, hence, a systematic approach to preventing novel pandemic emergence is required urgently. In this respect, artificial intelligence and machine learning are invaluable tools, not only for improving sensor reliability and the reproducibility of sensors, but also for gathering a large volume of information from biosensor responses and analyzing it to create epidemiological models to predict potential pandemics and disease.

In future studies, researchers can focus on developing user-friendly and low-cost biosensors that individuals can perform the tests at home to detect different viruses with a single integrated platform. Another crucial parameter that can be optimized is the detection time, as a person infected with certain viruses, such as COVID-19, can take two to seven days to display symptoms, allowing the virus to be easily transmitted from a patient to a healthy person. Future studies can also concentrate on easy manufacturing label-free biosensors to eliminate the need for harsh reagent storage, or they could develop novel reagents and integrate them into the biosensor structure.

References

- [1] Binnig G, Quate C F and Gerber C 1986 Atomic force microscope *Phys. Rev. Lett.* **56** 930
- [2] Datar R *et al* 2009 Cantilever sensors: nanomechanical tools for diagnostics *MRS Bull.* **34** 449–54
- [3] Ilic B, Yang Y and Craighead H G 2004 Virus detection using nanoelectromechanical devices *Appl. Phys. Lett.* **85** 2604–6
- [4] Craighead H G 2000 Nanoelectromechanical systems *Science* **290** 1532–5
- [5] Basu A K, Basu A and Bhattacharya S 2020 Micro/nano fabricated cantilever based biosensor platform: a review and recent progress *Enzyme Microb. Technol.* **139** 109558
- [6] Kim T S, Yoon D S and Lee J H 2006 Nanomechanical cantilever devices for biological sensors *Micromanufacturing and Nanotechnology* (Berlin: Springer)
- [7] Squires T M, Messinger R J and Manalis S 2008 Making it stick: convection, reaction and diffusion in surface-based biosensors *Nat. Biotechnol.* **26** 417–26
- [8] Kirstein K U *et al* 2005 Cantilever-based biosensors in CMOS technology *Proc. Design, Automation and Test in Europe DATE '05* pp 1340–1
- [9] Ziegler C 2004 Cantilever-based biosensors *Anal. Bioanal. Chem.* **379** 946–59
- [10] Yao D, Fu J and Li F 2021 Measurement of quasi-static and dynamic mechanical properties of soft materials based on instrumented indentation using a piezoelectric cantilever *Polym. Test.* **96** 107056

- [11] Thuau D, Ducrot P-H, Poulin P, Dufour I and Ayela C 2018 Integrated electromechanical transduction schemes for polymer MEMS sensors *Micromachines*. **9** 197
- [12] Yang H and Gijs M A M 2018 Micro-optics for microfluidic analytical applications *Chem. Soc. Rev.* **47** 1391–458
- [13] Enning R, Ziegler D, Nievergelt A, Friedlos R, Venkataramani K and Stemmer A 2011 A high frequency sensor for optical beam deflection atomic force microscopy *Rev. Sci. Instrum.* **82** 43705
- [14] Pfreundschuh M, Martinez-Martin D, Mulvihill E, Wegmann S and Muller D J 2014 Multiparametric high-resolution imaging of native proteins by force-distance curve-based AFM *Nat. Protoc.* **9** 1113–30
- [15] Johnson B N and Mutharasan R 2012 Sample preparation-free, real-time detection of microRNA in human serum using piezoelectric cantilever biosensors at attomole level *Anal. Chem.* **84** 10426–36
- [16] Mishra R and Hegner M 2014 Effect of non-specific species competition from total RNA on the static mode hybridization response of nanomechanical assays of oligonucleotides *Nanotechnology* **25** 225501
- [17] Basu A K, Sah A N, Pradhan A and Bhattacharya S 2019 Poly-L-Lysine functionalised MWCNT-rGO nanosheets based 3-d hybrid structure for femtomolar level cholesterol detection using cantilever based sensing platform *Sci. Rep.* **9** 1–13
- [18] Bouchaala A, Nayfeh A H and Younis M I 2016 Frequency shifts of micro and nano cantilever beam resonators due to added masses *J. Dyn. Syst. Meas. Control Trans. ASME*. **138**
- [19] Martinazzo J, Brezolin A and Steffens C 2020 21st century nanoscience—a handbook: nanopharmaceuticals, nanomedicine, and food nanoscience *Detection of Pesticides Using Cantilever Nanobiosensors* (Boca Raton, FL: CRC Press)
- [20] Xie D, Chen D, Peng S, Yang Y, Xu L and Wu F 2019 A low power cantilever-based metal oxide semiconductor gas sensor *IEEE Electron Device Lett.* **40** 1178–81
- [21] Agarwal D K, Maheshwari N, Mukherji S and Rao V R 2016 Asymmetric immobilization of antibodies on a piezo-resistive micro-cantilever surface *RSC Adv.* **6** 17606–16
- [22] Bai X, Hou H, Zhang B and Tang J 2014 Label-free detection of kanamycin using aptamer-based cantilever array sensor *Biosens. Bioelectron.* **56** 112–6
- [23] Chen Y, Xu P and Li X 2010 Self-assembling siloxane bilayer directly on SiO₂ surface of micro-cantilevers for long-term highly repeatable sensing to trace explosives *Nanotechnology* **21** 265501
- [24] Andrade M A *et al* 2020 A nanomechanical genosensor using functionalized cantilevers to detect the cancer biomarkers miRNA-203 and miRNA-205 *IEEE Sens. J.* **20** 2860–7
- [25] Nyang'au W O, Setiono A, Schmidt A, Bosse H and Peiner E 2020 Sampling and mass detection of a countable number of microparticles using on-cantilever imprinting *Sensors* **20** 2508
- [26] Gad-el-Hak M 2001 *The MEMS Handbook* (Boca Raton, FL: CRC Press)
- [27] Beeby S, Ensel G, White N and Kraft M 2004 *MEMS Mechanical Sensors* (Boston, MA: Artech House)
- [28] Riesch C, Keplinger F, Reichel E K and Jakoby B 2006 Characterizing resonating cantilevers for liquid property sensing *Proc. IEE Sensors* pp 1070–3
- [29] Brand O, Dufour I, Heinrich S M and Josse F 2015 Resonant MEMS: principles, modeling, implementation, and applications *Resonant MEMS: Principles, Modeling, Implementation, and Applications*. (New York: Wiley) pp 1–483
- [30] Sader J E 1998 Frequency response of cantilever beams immersed in viscous fluids with applications to the atomic force microscope *J. Appl. Phys.* **84** 64–76

- [31] Van Eysden C A and Sader J E 2009 Frequency response of cantilever beams immersed in compressible fluids with applications to the atomic force microscope *J. Appl. Phys.* **106** 094904
- [32] Raiteri R, Grattarola M, Butt H J and Skládal P 2001 Micromechanical cantilever-based biosensors *Sensors Actuators B* **79** 115–26
- [33] Shiraishi N, Ikehara T, Dao D V, Sugiyama S and Ando Y 2013 Fabrication and testing of polymer cantilevers for VOC sensors *Sensors Actuators A* **02** 233–9
- [34] Abadal G *et al* 2001 Electromechanical model of a resonating nano-cantilever-based sensor for high-resolution and high-sensitivity mass detection *Nanotechnology* **12** 100
- [35] Durdaut P *et al* 2020 Fundamental noise limits and sensitivity of piezoelectrically driven magnetoelastic cantilevers *J. Microelectromech. Syst.* **29** 1347–61
- [36] Teva J *et al* 2004 On the electromechanical modelling of a resonating nano-cantilever-based transducer *Ultramicroscopy* (Amsterdam: North-Holland) pp 225–32
- [37] Park J, Nishida S, Lambert P, Kawakatsu H and Fujita H 2011 High-resolution cantilever biosensor resonating at air-liquid in a microchannel *Lab Chip*. **11** 4187–93
- [38] Yang Y-T, Callegari C, Feng X L, Ekinci K L and Roukes M L 2006 Zeptogram-scale nanomechanical mass sensing *Nano Lett.* **6** 583–6
- [39] Ilic B, Yang Y, Aubin K, Reichenbach R, Krylov S and Craighead H G 2005 Enumeration of DNA molecules bound to a nanomechanical oscillator *Nano Lett.* **5** 925–9
- [40] Fu Y Q *et al* 2017 Advances in piezoelectric thin films for acoustic biosensors, acousto-fluidics and lab-on-chip applications *Prog. Mater. Sci.* **89** 31–91
- [41] Sauerbrey G 1959 Use of quartz vibrator for weighting thin films on a microbalance *Z. Phys.* **155** 206–12
- [42] Zhang J, Zhang X, Wei X, Xue Y, Wan H and Wang P 2021 Recent advances in acoustic wave biosensors for the detection of disease-related biomarkers: a review *Anal Chim Acta* **1164** 338321
- [43] Murillo A E *et al* 2019 Development of AlN thin films for breast cancer acoustic biosensors *J. Mater. Res. Technol.* **8** 350–8
- [44] Fogel R *et al* 2019 Bulk and surface acoustic wave sensor arrays for multi-analyte detection: a review *Biosens. Bioelectron.* **163** 112164
- [45] Zhang Y, Luo J, Flewitt A J, Cai Z and Zhao X 2018 Film bulk acoustic resonators (FBARs) as biosensors: a review *Biosens. Bioelectron.* **116** 1–15
- [46] Afzal A, Mujahid A, Schirhagl R, Bajwa S Z, Latif U and Feroz S 2017 Gravimetric viral diagnostics: QCM based biosensors for early detection of viruses *Chemosensors*. **5** 7
- [47] Dey N, Ashour A S, Mohamed W S and Nguyen N G 2019 Acoustic sensors *Acoustic Sensors for Biomedical Applications* (Berlin: Springer) pp 33–41
- [48] Salouti M and Derakhshan F K 2020 Biosensors and nanobiosensors in environmental applications *Biogenic Nano-Particles and their Use in Agro-Ecosystems* (Berlin: Springer) pp 515–91
- [49] Pirinçi Ş, Ertekin Ö, Laguna D E, Özen F, Öztürk Z Z and Öztürk S 2018 Label-free QCM immunosensor for the detection of ochratoxin A *Sensors* **18** 1161
- [50] Yu X, Chen F, Wang R and Li Y 2018 Whole-bacterium SELEX of DNA aptamers for rapid detection of *E.coli* O157:H7 using a QCM sensor *J. Biotechnol.* **266** 39–49
- [51] Yilmaz E, Majidi D, Ozgur E and Denizli A 2015 Whole cell imprinting based *Escherichia coli* sensors: a study for SPR and QCM *Sensors Actuators B* **209** 714–21
- [52] Wang R, Wang L, Callaway Z T, Lu H, Huang T J and Li Y 2017 A nanowell-based QCM aptasensor for rapid and sensitive detection of avian influenza virus *Sensors Actuators B* **240** 934–40

- [53] Wang R and Li Y 2013 Hydrogel based QCM aptasensor for detection of avian influenzavirus *Biosens. Bioelectron.* **42** 148–55
- [54] Arif S, Qudsia S, Urooj S, Chaudry N, Arshad A and Andleeb S 2015 Blueprint of quartz crystal microbalance biosensor for early detection of breast cancer through salivary autoantibodies against ATP6AP1 *Biosens. Bioelectron.* **65** 62–70
- [55] Liu J *et al* 2020 A microfabricated thickness shear mode electroacoustic resonator for the label-free detection of cardiac troponin in serum *Talanta* **215** 120890
- [56] Mujahid A, Afzal A and Dickert F L 2019 An overview of high frequency acoustic sensors —QCMs, SAWs and FBARs—chemical and biochemical applications *Sensors* **19** 4395
- [57] Ren J, Chu H, Bai Y, Wang R, Chen P and Chen J 2021 Research and design of high sensitivity FBAR micro-mass sensors *IOP Conf. Series: Earth and Environmental Science (Bristol) (IOP Publishing)* pp 42014
- [58] Liu C, Pang W, Duan X and Wang Y 2020 Dual functions of GHz frequency acoustic resonator system for biosamples capture and sensing 2020 42nd Annual Int. Conf. of the IEEE Engineering in Medicine & Biology Society (EMBC) (Piscataway, NJ) (IEEE) pp 3994–7
- [59] White R M and Voltmer F W 1965 Direct piezoelectric coupling to surface elastic waves *Appl. Phys. Lett.* **7** 314–6
- [60] Xu Z and Yuan Y J 2018 Implementation of guiding layers of surface acoustic wave devices: a review *Biosens. Bioelectron.* **99** 500–12
- [61] Raj V B, Singh H, Nimal A T, Sharma M U and Gupta V 2013 Oxide thin films (ZnO, TeO₂, SnO₂, and TiO₂) based surface acoustic wave (SAW) E-nose for the detection of chemical warfare agents *Sensors Actuators B* **178** 636–47
- [62] Constantinoiu I and Viespe C 2019 Detection of volatile organic compounds using surface acoustic wave sensor based on nanoparticles incorporated in polymer *Coatings*. **9** 373
- [63] Chen C and Jin J 2020 Surface acoustic wave vapor sensor with graphene interdigital transducer for TNT detection *Sens. Imag.* **21** 1–12
- [64] Al-Mashat L, Tran H D, Wlodarski W, Kaner R B and Kalantar-Zadeh K 2008 Polypyrrole nanofiber surface acoustic wave gas sensors *Sensors Actuators B* **134** 826–31
- [65] Kidakova A, Boroznjak R, Reut J, Öpik A, Saarma M and Syritski V 2020 Molecularly imprinted polymer-based SAW sensor for label-free detection of cerebral dopamine neurotrophic factor protein *Sensors Actuators B* **308** 127708
- [66] Wang K *et al* 2018 Sorting of tumour cells in a microfluidic device by multi-stage surface acoustic waves *Sensors Actuators B* **258** 1174–83
- [67] Go D B, Atashbar M Z, Ramshani Z and Chang H-C 2017 Surface acoustic wave devices for chemical sensing and microfluidics: a review and perspective *Anal. Methods* **9** 4112–34
- [68] Lamanna L, Rizzi F, Bhethanabotla V R and De Vittorio M 2020 Conformable surface acoustic wave biosensor for *E.coli* fabricated on PEN plastic film *Biosens. Bioelectron.* **163** 112164
- [69] Bisoffi M *et al* 2008 Detection of viral bioagents using a shear horizontal surface acoustic wave biosensor *Biosens. Bioelectron.* **23** 1397–403
- [70] Luo J, Quan A, Fu C and Li H 2017 Shear-horizontal surface acoustic wave characteristics of a (110) ZnO/SiO₂/Si multilayer structure *J. Alloys Compd.* **693** 558–64
- [71] Lange K, Rapp B E and Rapp M 2008 Surface acoustic wave biosensors: a review *Anal. Bioanal. Chem.* **391** 1509–19
- [72] Puiu M, Gurban A-M, Rotariu L, Brajnicov S, Viespe C and Bala C 2015 Enhanced sensitive Love wave surface acoustic wave sensor designed for immunoassay formats *Sensors* **15** 10511–25

- [73] Devkota J, Ohodnicki P R and Greve D W 2017 SAW sensors for chemical vapors and gases *Sensors* **17** 801
- [74] Mohseni H and Ng C-T 2019 Rayleigh wave propagation and scattering characteristics at debondings in fibre-reinforced polymer-retrofitted concrete structures *Struct. Heal. Monit.* **18** 303–17
- [75] Agostini M, Greco G and Cecchini M 2018 A Rayleigh surface acoustic wave (R-SAW) resonator biosensor based on positive and negative reflectors with sub-nanomolar limit of detection *Sensors Actuators B* **254** 1–7
- [76] Pantazis A K, Konstantinidis G and Gizeli E 2016 Study of the effect of the operating frequency of a GaN Lamb wave device to viscosity and protein sensing *IEEE Sens. J.* **16** 7028–36
- [77] Nam J, Jang W S, Kim J, Lee H and Lim C S 2019 Lamb wave-based molecular diagnosis using DNA hydrogel formation by rolling circle amplification (RCA) process *Biosens. Bioelectron* **142** 111496
- [78] Hamidullah M, Élie-Caille C and Leblois T 2020 Theoretical study and finite element simulation of ZnO/GaAs higher-order Lamb waves for microsensor application in liquid media *IEEE Sensors* (Piscataway, NJ: IEEE) pp 1–4
- [79] Uchino K and Debus J C 2012 *Applications of ATILA FEM Software to Smart Materials: Case Studies in Designing Devices*. (Cambridge: Woodhead) pp 1–386
- [80] Curry E J *et al* 2018 Biodegradable piezoelectric force sensor *Proc. Natl Acad. Sci.* **115** 909–14
- [81] Lee M *et al* 2012 A hybrid piezoelectric structure for wearable nanogenerators *Adv. Mater.* **24** 1759–64
- [82] Su L, Fong C C, Cheung P Y and Yang M 2017 Development of novel piezoelectric biosensor using PZT ceramic resonator for detection of cancer markers *Methods in Molecular Biology* (Totowa, NJ: Humana Press) pp 277–91
- [83] Zhu B *et al* 2017 Self-focused AlScN film ultrasound transducer for individual cell manipulation *ACS Sens.* **2** 172–7
- [84] Rajala S *et al* 2016 Cellulose nanofibril film as a piezoelectric sensor material *ACS Appl. Mater. Interfaces* **8** 15607–14
- [85] Ghosh S K and Mandal D 2017 Sustainable energy generation from piezoelectric biomaterial for noninvasive physiological signal monitoring *ACS Sustain Chem Eng.* **5** 8836–43
- [86] Ghosh S K and Mandal D 2017 Bio-assembled, piezoelectric prawn shell made self-powered wearable sensor for non-invasive physiological signal monitoring *Appl. Phys. Lett.* **110** 123701
- [87] Pohanka M 2018 Piezoelectric biosensor for the determination of tumor necrosis factor alpha *Talanta* **178** 970–3
- [88] Chorsi M T *et al* 2019 Piezoelectric biomaterials for sensors and actuators *Adv. Mater.* **31** 1–15
- [89] Janshoff A, Galla H J and Steinem C 2000 Piezoelectric mass-sensing devices as biosensors – an alternative to optical biosensors? *Angew Chem. – Int. Ed.* **39** 4004–32
- [90] Ramadan K S, Sameoto D and Evoy S 2014 A review of piezoelectric polymers as functional materials for electromechanical transducers *Smart Mater. Struct.* **23** 033001
- [91] Algarni A S *et al* 2021 A review of actuation and sensing mechanisms in MEMS-based sensor devices *Nanoscale Res. Lett.* **16** 16
- [92] Pang W, Zhao H, Kim E S, Zhang H, Yu H and Hu X 2012 Piezoelectric micro-electromechanical resonant sensors for chemical and biological detection *Lab Chip* **12** 29–44

- [93] Huang I, Lee M, Hsu C and Wang C 2012 Chemical development of a flexural plate-wave (FPW) immunoglobulin-E (IgE) allergy bio-sensing microsystem *Sensors Actuators B* **162** 184–93
- [94] Jung J, Lee W, Kang W, Shin E, Ryu J and Choi H 2017 Review of piezoelectric micromachined ultrasonic transducers and their applications *J Micromech. Microeng.* **27** 113001
- [95] Pohanka M 2018 Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications *Materials (Basel)* **11** 448
- [96] Zhang J X J and Hoshino K 2019 Mechanical transducers: cantilevers, acoustic wave sensors, and thermal sensors *Molecular Sensors and Nanodevices* (Amsterdam: Elsevier) pp 311–412
- [97] Fritz J *et al* 2000 Translating biomolecular recognition into nanomechanics *Science* **288** 316–8
- [98] Gibbs J W and Josiah W 1839–903 *Bumstead HA, 1870-920, Van Name RG, 1877-. Scientific Papers of J. Willard Gibbs* (London: Longmans, Green and Co.) 1906
- [99] Thundat T, Oden P I and Warmack R J 1997 Microcantilever sensors *Microscale Thermophys. Eng.* **1** 185–99
- [100] Butt H J 1996 A sensitive method to measure changes in the surface stress of solids *J. Colloid Interface Sci.* **180** 251–60
- [101] Mathew R, Ravi and Sankar A 2018 A review on surface stress-based miniaturized piezoresistive SU-8 polymeric cantilever sensors *Nano-Micro Letters* vol 10 (Berlin: Springer) pp 1–41
- [102] Stoney G G 1909 The tension of metallic films deposited by electrolysis *Proc. R Soc. London Ser. A* **82** 172–5
- [103] Liangruksa M 2008 *Effect of Surface Stress on Micromechanical Cantilevers for Sensing Applications*. (Blacksburg, VA: Virginia Tech)
- [104] Sang S, Zhao Y, Zhang W, Li P, Hu J and Li G 2014 Surface stress-based biosensors *Biosens. Bioelectron.* **51** 124–35
- [105] Lundbæk J A, Maer A M and Andersen O S 1997 Lipid bilayer electrostatic energy, curvature stress, and assembly of gramicidin channels *Biochemistry.* **36** 5695–701
- [106] Berger R *et al* 1997 Surface stress in the self-assembly of alkanethiols on gold *Science* **276** 2021–4
- [107] Jian A *et al* 2017 A PDMS surface stress biosensor with optimized micro-membrane: fabrication and application *Sensors Actuators B* **242** 969–76
- [108] Takahashi K, Oyama H, Misawa N, Okumura K, Ishida M and Sawada K 2013 Surface stress sensor using MEMS-based Fabry–Perot interferometer for label-free biosensing *Sensors Actuators B* **188** 393–9
- [109] Yue M *et al* 2004 A 2-D microcantilever array for multiplexed biomolecular analysis *J. Microelectromech. Syst.* **13** 290–9
- [110] Marie R, Jensenius H, Thaysen J, Christensen C B and Boisen A 2002 Adsorption kinetics and mechanical properties of thiol-modified DNA-oligos on gold investigated by microcantilever sensors *Ultramicroscopy* **91** 29–36
- [111] Varshney M, Li Y, Srinivasan B and Tung S 2007 A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157:H7 in food samples *Sensors Actuators B* **128** 99–107
- [112] Choi Y J, Takahashi T, Taki M, Sawada K and Takahashi K 2021 Label-free attomolar protein detection using a MEMS optical interferometric surface-stress immunosensor with a freestanding PMMA/parylene-C nanosheet *Biosens. Bioelectron.* **172** 112778

- [113] Mertens J *et al* 2008 Label-free detection of DNA hybridization based on hydration-induced tension in nucleic acid films *Nat. Nanotechnol.* **3** 301–7
- [114] Godin M *et al* 2004 Surface stress, kinetics, and structure of alkanethiol self-assembled monolayers *Langmuir* **20** 7090–6
- [115] Battiston F M *et al* 2001 A chemical sensor based on a microfabricated cantilever array with simultaneous resonance-frequency and bending readout *Sensors Actuators B* **77** 122–31
- [116] Lim Y, Kouzani A and Duan W *et al* 2014 A surface-stress-based microcantilever aptasensor *IEEE Trans. on Biomed. Circ. Sys.* **8** 15–24
- [117] Yue M 2004 *Multiplexed Label-Free Bioassays Based on Nanomechanics and Nanofluidics* (Ann Arbor, MI: ProQuest Dissertations Publishing)
- [118] Zhang W *et al* 2013 Structural optimization of the micro-membrane for a novel surface stress-based capacitive biosensor *Microelectron. Eng.* **106** 9–12
- [119] Benderitter M, Vincent-Genod L, Pouget J P and Voisin P 2003 The cell membrane as a biosensor of oxidative stress induced by radiation exposure: a multiparameter investigation *Radiat. Res.* **159** 471–83
- [120] Zhao D *et al* 2019 Sandwich-type surface stress biosensor based on self-assembled gold nanoparticles in PDMS film for BSA detection *ACS Biomater. Sci. Eng.* **5** 6274–80
- [121] Sang S and Witte H 2010 A novel PDMS micro membrane biosensor based on the analysis of surface stress *Biosens. Bioelectron.* **25** 2420–4
- [122] Yang S M and Chang C 2010 A piezoresistive bridge-microcantilever biosensor by CMOS process for surface stress measurement *Sensors Actuators B* **145** 405–10
- [123] Zhao D *et al* 2019 Surface stress-based biosensor with stable conductive AuNPs network for biomolecules detection *Appl. Surf. Sci.* **491** 443–50
- [124] Doelle M, Mager D, Ruther P and Paul O 2006 Geometry optimization for planar piezoresistive stress sensors based on the pseudo-Hall effect *Sensors Actuators A* **127** 261–9
- [125] Takahashi T, Hizawa T, Misawa N, Taki M, Sawada K and Takahashi K 2018 Surface stress sensor based on MEMS Fabry–Perot interferometer with high wavelength selectivity for label-free biosensing *J. Micromech. Microeng.* **28** 054002
- [126] Yoshikawa G, Akiyama T, Gautsch S, Vettiger P and Rohrer H 2011 Nanomechanical membrane-type surface stress sensor *Nano Lett.* **11** 1044–8
- [127] Jones K E *et al* 2008 Global trends in emerging infectious diseases *Nature* **451** 990–3
- [128] Qureshi A and Niazi J H 2020 Biosensors for detecting viral and bacterial infections using host biomarkers: a review *Analyst* **145** 7825–48
- [129] Narita F *et al* 2021 A review of piezoelectric and magnetostrictive biosensor materials for detection of COVID-19 and other viruses *Adv. Mater.* **33** 2005448
- [130] Rodovalho V, Alves L, Castro A, Madurro J, Brito-Madurro A and Santos A 2015 Biosensors applied to diagnosis of infectious diseases—an update *Austin J. Biosens. Bioelectron.* **1** 1–12
- [131] Sin M L Y, Mach K E, Wong P K and Liao J C 2014 Advances and challenges in biosensor-based diagnosis of infectious diseases *Expert Rev. Mol. Diagn.* **14** 225–44
- [132] Song Z *et al* 2019 From SARS to MERS, thrusting coronaviruses into the spotlight *Viruses.* **11** 59
- [133] Yadav S *et al* 2021 SERS based lateral flow immunoassay for point-of-care detection of SARS-CoV-2 in clinical samples *ACS Appl Bio Mater.* **4** 2974–95
- [134] Zhong N S *et al* 2020 Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People’s Republic of China, in February, 2003 Elsevier Connect, the company’s public news and information *Lancet* **362** 1353–8

- [135] Alyami M H, Alyami H S and Warraich A 2020 Middle East Respiratory Syndrome (MERS) and novel coronavirus disease-2019 (COVID-19): from causes to preventions in Saudi Arabia *Saudi Pharm J.* **28** 1481–91
- [136] Butowt R and Bilinska K 2020 SARS-CoV-2: olfaction, brain infection, and the urgent need for clinical samples allowing earlier virus detection *ACS Chem. Neurosci.* **11** 1200–3
- [137] Chan J F W *et al* 2020 A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster *Lancet* **395** 514–23
- [138] Li T *et al* 2021 Computer-aided diagnosis of COVID-19 CT scans based on spatiotemporal information fusion *J. Healthc. Eng.* **2021** 6649591
- [139] Ruuskanen O, Lahti E, Jennings L C and Murdoch D 2011 Viral pneumonia *Lancet* **377** 1264–75
- [140] Cui F and Zhou H S 2020 Diagnostic methods and potential portable biosensors for coronavirus disease 2019 *Biosens. Bioelectron.* **165** 112349
- [141] Liu W *et al* 2020 Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2 *J. Clin. Microbiol.* **58** 6
- [142] Lu R, Wu X, Wan Z, Li Y, Jin X and Zhang C 2020 A novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2 *Int. J. Mol. Sci.* **21** 2826
- [143] Xia N, Wang G and Gong W 2020 Serological test is an efficient supplement for detecting RNA to confirm SARS-CoV-2 infection *Preprint.* 1–6
- [144] Broughton J P *et al* 2020 Rapid detection of 2019 novel coronavirus SARS-CoV-2 using a CRISPR-based DETECTR lateral flow assay *medRxiv* **415** 1–28
- [145] Fang Y and Pang P 2020 Senivity of chest CT for COVID-19: comparasion to RT-PCR *Radiology* **296** 15–7
- [146] Pan Y *et al* 2020 Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients *J. Infect.* **81** e28–32
- [147] Wang Y, Kang H, Liu X and Tong Z 2020 Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak *J. Med. Virol.* **92** 538–9
- [148] Ranjan P *et al* 2020 Biosensor-based diagnostic approaches for various cellular biomarkers of breast cancer: a comprehensive review *Anal. Biochem.* **610** 113996
- [149] Demeke Teklemariam A, Samaddar M, Alharbi M G, Al-Hindi R R and Bhunia A K 2020 Biosensor and molecular-based methods for the detection of human coronaviruses: a review *Mol. Cell. Probes* **54** 1–8
- [150] Zuo B, Li S, Guo Z, Zhang J and Chen C 2004 Piezoelectric immunosensor for SARS-associated coronavirus in sputum *Anal. Chem.* **76** 3536–40
- [151] Velanki S and Ji H F 2006 Detection of feline coronavirus using microcantilever sensors *Meas. Sci. Technol.* **17** 2964–8
- [152] Du F and Zhang F 2020 Application of novel MEMS sensor in airborne COVID-19 virus detection *Sci. J. Info. Eng.* **9** 1–5
- [153] Deeks S G, Overbaugh J, Phillips A and Buchbinder S 2015 HIV infection *Nat. Rev. Dis. Prim.* **1**
- [154] World Health Organization HIV/AIDS (<https://who.int/news-room/fact-sheets/detail/hiv-aids>) (Accessed: on 4th April 2021)
- [155] Lam Y, Abu-Lail N I, Alam M S and Zauscher S 2006 Using microcantilever deflection to detect HIV-1 envelope glycoprotein gp120 *Nanomed. Nanotech. Biol Med.* **2** 222–9
- [156] Sheikh S, Blaszykowski C and Thompson M 2011 Label-free detection of HIV-2 antibodies in serum with an ultra-high frequency acoustic wave sensor *Talanta* **85** 816–9

- [157] Lu C H *et al* 2012 Sensing HIV related protein using epitope imprinted hydrophilic polymer coated quartz crystal microbalance *Biosens. Bioelectron.* **31** 439–44
- [158] Ly T N, Park S and Park S J 2016 Detection of HIV-1 antigen by quartz crystal microbalance using gold nanoparticles *Sensors Actuators B* **237** 452–8
- [159] Bisoffi M, Severns V, Branch D W, Edwards T L and Larson R S 2013 Rapid detection of human immunodeficiency virus types 1 and 2 by use of an improved piezoelectric biosensor *J. Clin. Microbiol.* **51** 1685–91
- [160] Saurabh N K, Deekshanya B and Rudraswamy S B 2017 Design of microcantilever sensor for detection of P24 antigen in HIV diagnoses *Int. J. Sci. Eng. Sci.* **1**
- [161] Zhou J *et al* 2017 Assessing activity of hepatitis A virus 3C protease using a cyclized luciferase-based biosensor *Biochem. Biophys. Res. Commun.* **488** 621–7
- [162] Shepard C W, Simard E P, Finelli L, Fiore A E and Bell B P 2006 Hepatitis B virus infection: epidemiology and vaccination *Epidemiol Rev.* **28** 112–25
- [163] Donmez S, Arslan F and Arslan H 2015 A nucleic acid biosensor for detection of hepatitis C virus genotype 1a using poly(L-Glutamic acid)-modified electrode *Appl. Biochem. Biotechnol.* **176** 1431–44
- [164] Donmez S, Arslan F and Arslan H 2016 Sequence-specific label-free nucleic acid biosensor for the detection of the hepatitis C virus genotype 1a using a disposable pencil graphite electrode *Artif. Cells, Nanomed. Biotechnol.* **44** 912–7
- [165] Shawky S M, Awad A M, Allam W, Alkordi M H and EL-Khamisy S F 2017 Gold aggregating gold: a novel nanoparticle biosensor approach for the direct quantification of hepatitis C virus RNA in clinical samples *Biosens. Bioelectron.* **92** 349–56
- [166] Nainan O V, Xia G, Vaughan G and Margolis H S 2006 Diagnosis of hepatitis A virus infection: a molecular approach *Clin Microbiol Rev.* **19** 63–79
- [167] Wang X *et al* 2010 Gold nanorod-based localized surface plasmon resonance biosensor for sensitive detection of hepatitis B virus in buffer, blood serum and plasma *Biosens. Bioelectron.* **26** 404–10
- [168] Mathiesen L R, Feinstone S M, Wong D C, Skinhoj P and Purcell R H 1978 Enzyme-linked immunosorbent assay for detection of hepatitis A antigen in stool and antibody to hepatitis A antigen in sera: comparison with solid-phase radioimmunoassay, immune electron microscopy, and immune adherence hemagglutination assay *J. Clin. Microbiol.* **7** 184–93
- [169] Gocke D J and Kachani Z F 1973 Solid-phase radioimmunoassay for hepatitis B antigen *JAMA J. Am. Med. Assoc.* **224** 1425–6
- [170] Shen J *et al* 2015 Immunochromatographic assay for quantitative and sensitive detection of hepatitis B virus surface antigen using highly luminescent quantum dot-beads *Talanta* **142** 145–9
- [171] Sabouri S, Ghourchian H, Shourian M and Boutorabi M 2014 A gold nanoparticle-based immunosensor for the chemiluminescence detection of the hepatitis B surface antigen *Anal. Methods* **6** 5059–66
- [172] Ryu J H *et al* 2018 Development of a rapid automated fluorescent lateral flow immunoassay to detect hepatitis B surface antigen (HBsAg), antibody to HBsAg, and antibody to hepatitis C *Ann Lab Med.* **38** 578
- [173] Peruski A H and Peruski L F 2003 Immunological methods for detection and identification of infectious disease and biological warfare agents *Clin Diagn Lab Immunol.* **10** 506–13
- [174] Zhang C *et al* 2021 Rapid one-pot detection of SARS-CoV-2 based on a lateral flow assay in clinical samples *Anal. Chem.* **93** 3325–30
- [175] Cobo F 2012 Application of molecular diagnostic techniques for viral testing *Open Virol J.* **5** 104–14

- [176] Khodakov D A, Zakharova N V, Gryadunov D A, Filatov F P, Zasedatelev A S and Mikhailovich V M 2008 An oligonucleotide microarray for multiplex real-time PCR identification of HIV-1, HBV, and HCV *Biotechniques*. **44** 241–8
- [177] de Vera M E *et al* 2018 Transplantation of hepatitis C virus (HCV) antibody positive, nucleic acid test negative donor kidneys to HCV negative patients frequently results in seroconversion but not HCV viremia *Am. J. Transplant.* **18** 2451–6
- [178] Narayanamurthy V, Jeroish Z E, Bhuvaneshwari K S and Samsuri F 2021 Hepatitis C virus (HCV) diagnosis: via microfluidics *Anal. Methods* **13** 740–63
- [179] Parizad E and Khosravi A 2012 Detection of HBV DNA in cerumen and sera of Hbsag negative patients with chronic hepatitis B infection *Iran Red Crescent* **14** 186–7
- [180] Srisomwat C, Teengam P, Chuaypen N, Tangkijvanich P, Vilaivan T and Chailapakul O 2020 Pop-up paper electrochemical device for label-free hepatitis B virus DNA detection *Sensors Actuators B* **316** 128077
- [181] Yoo H, Shin J, Sim J, Cho H and Hong S 2020 Biosensors and bioelectronics reusable surface plasmon resonance biosensor chip for the detection of H1N1 influenza virus *Biosens. Bioelectron.* **168** 112561
- [182] Perumal V and Hashim U 2014 Advances in biosensors: principle, architecture and applications *J. Appl. Biomed.* **12** 1–15
- [183] Liu Y P and Yao C Y 2015 Rapid and quantitative detection of hepatitis B virus *World J Gastroenterol.* **21** 11954–63
- [184] Lim H J, Saha T, Tey B T, Tan W S and Ooi C W 2020 Quartz crystal microbalance-based biosensors as rapid diagnostic devices for infectious diseases *Biosens. Bioelectron.* **168** 112513
- [185] Zhou X, Liu L, Hu M, Wang L and Hu J 2002 Detection of hepatitis B virus by piezoelectric biosensor *J. Pharm. Biomed. Anal.* **27** 341–5
- [186] Yao C *et al* 2008 Hybridization assay of hepatitis B virus by QCM peptide nucleic acid biosensor *Biosens. Bioelectron.* **23** 879–85
- [187] Zhao J *et al* 2021 Gold nanoparticles amplified microcantilever biosensor for detecting protein biomarkers with high sensitivity *Sensors Actuators A* **321** 112563
- [188] Wu G 2001 Origin of nanomechanical cantilever motion generated from biomolecular interactions *Proc. Natl Acad. Sci.* **98** 1560–4
- [189] Wu G, Datar R H, Hansen K M, Thundat T, Cote R J and Majumdar A 2001 Bioassay of prostate-specific antigen (PSA) using microcantilevers *Nat. Biotechnol.* **19** 856–60
- [190] Timurdogan E, Alaca B E, Kavakli I H and Urey H 2011 MEMS biosensor for detection of hepatitis A and C viruses in serum *Biosens. Bioelectron.* **28** 189–94
- [191] Cha B H *et al* 2009 Detection of hepatitis B virus (HBV) DNA at femtomolar concentrations using a silica nanoparticle-enhanced microcantilever sensor *Biosens. Bioelectron.* **25** 130–5
- [192] Lee H J, Namkoong K, Cho E C, Ko C, Park J C and Lee S S 2009 Surface acoustic wave immunosensor for real-time detection of hepatitis B surface antibodies in whole blood samples *Biosens. Bioelectron.* **24** 3120–5
- [193] Xu T, Miao J, Wang Z, Yu L and Li C M 2011 Micro-piezoelectric immunoassay chip for simultaneous detection of hepatitis B virus and α -fetoprotein *Sensors Actuators B* **151** 370–6
- [194] WHO Ebola Response Team 2014 Ebola virus disease in West Africa—The first 9 months of the epidemic and forward projections *N. Engl. J. Med.* **371** 1481–95
- [195] Beeching N J, Fenech M and Houlihan C F 2014 Ebola virus disease *BMJ (Online)* **349** g7348

- [196] Mahanty S and Bray M 2004 Pathogenesis of filoviral haemorrhagic fevers *The Lancet Infect. Dis.* **4** 487–98
- [197] Pourrut X *et al* 2005 The natural history of Ebola virus in Africa *Microbes Infect.* **7** 1005–14
- [198] WHO 2021 Ebola Virus Disease. (<https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease>)
- [199] Chertow D S, Kleine C, Edwards J K, Scaini R, Giuliani R and Sprecher A 2014 Ebola virus disease in West Africa – clinical manifestations and management *N. Engl. J. Med.* **371** 2054–7
- [200] Brangel P *et al* 2018 A serological point-of-care test for the detection of IgG antibodies against Ebola virus in human survivors *ACS Nano.* **12** 63–73
- [201] Wu S *et al* 2016 An adenovirus vaccine expressing Ebola virus variant Makona glycoprotein is efficacious in guinea pigs and nonhuman primates *J. Infect. Dis.* **214** S326–32
- [202] Ksiazek T G, West C P, Rollin P E, Jahrling P B and Peters C J 1999 ELISA for the detection of antibodies to Ebola viruses *J Infect Dis.* **179** S192–8
- [203] Leroy E M *et al* 2000 Diagnosis of Ebola haemorrhagic fever by RT-PCR in an epidemic setting *J Med Virol.* **60** 463–7
- [204] Broadhurst M J, Brooks T J G and Pollock N R 2016 Diagnosis of ebola virus disease: past, present and future *Clin. Microb. Rev.* **29** 773–93
- [205] Chen Y *et al* 2017 Field-effect transistor biosensor for rapid detection of ebola antigen *Sci. Rep.* **7** 10974
- [206] Baca J, Severns V, Lovato D, Branch D and Larson R 2015 Rapid detection of Ebola virus with a reagent-free, point-of-care biosensor *Sensors* **15** 8605–14
- [207] Kuno G 2016 Zika virus *Mol. Detect. Hum. Viral Pathog.* **29** 313–20
- [208] Petersen L R, Jamieson D J, Powers A M and Honein M A 2016 Zika virus *New Engl. J. Med.* 1552–63
- [209] Musso D, Roche C, Nhan T X, Robin E, Teissier A and Cao-Lormeau V M 2015 Detection of Zika virus in saliva *J Clin Virol.* **68** 53–5
- [210] Dolai S and Tabib-Azar M 2020 433 MHz Lithium niobate microbalance aptamer-coated whole zika virus sensor with 370 Hz/ng sensitivity *IEEE Sens. J.* **20** 4269–74
- [211] Dolai S, Baker B and Tabib-Azar M 2021 Micro fabricated MEMS colorimetric devices to measure Zika-induced residual stress and mass loading *IEEE Sens. J.* **21** 4682–7
- [212] Marshall J A and Bruggink L D 2011 The dynamics of norovirus outbreak epidemics: recent insights *Int. J. Environ. Res. Public Health.* **8** 1141–9
- [213] Hall A J *et al* 2011 Centers for Disease Control & Prevention (CDC) updated norovirus outbreak management and disease prevention guidelines *Mortal Wkly. Rep. Recomm. Rep.* **60** 1–15
- [214] Kapikian A Z, Wyatt R G, Dolin R, Thornhill T S, Kalica A R and Chanock R M 1972 Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis *J Virol.* **10** 5
- [215] Bruggink L D and Marshall J A 2009 Norovirus epidemics are linked to two distinct sets of controlling factors *Int. J. Infect. Dis.* **13** e125–6
- [216] Robilotti E, Deresinski S and Pinsky B A 2015 Norovirus *Clin. Microb. Rev.* **28** 134–64
- [217] Chiba S *et al* 1979 An outbreak of gastroenteritis associated with calicivirus in an infant home *J. Med. Virol.* **4** 249–54
- [218] Rabenau H F, Stürmer M, Buxbaum S, Walczok A, Preiser W and Doerr H W 2003 Laboratory diagnosis of norovirus: which method is the best? *Intervirology.* **46** 232–8

- [219] Teunis P F M, Sukhrie F H A, Vennema H, Bogerman J, Beersma M F C and Koopmans M P G 2015 Shedding of norovirus in symptomatic and asymptomatic infections *Epidemiol. Infect.* **143** 1710–7
- [220] Chand R and Neethirajan S 2017 Microfluidic platform integrated with graphene-gold nano-composite aptasensor for one-step detection of norovirus *Biosens. Bioelectron.* **98** 47–53
- [221] Xiang L, Wang Z, Liu Z, Weigum S E, Yu Q and Chen M Y 2016 Inkjet-printed flexible biosensor based on graphene field effect transistor *IEEE Sens. J.* **16** 8359–64
- [222] Poddar S K 2002 Influenza virus types and subtypes detection by single step single tube multiplex reverse transcription-polymerase chain reaction (RT-PCR) and agarose gel electrophoresis *J. Virol. Methods* **99** 63–70
- [223] World Health Organization 2011 Manual for the laboratory diagnosis and virological surveillance of influenza *World Heal Organ.* **153**
- [224] Metzgar D *et al* 2010 Single assay for simultaneous detection and differential identification of human and avian influenza virus types, subtypes, and emergent variants *PLoS One.* **5** e8995
- [225] Peduru Hewa T M, Tannock G A, Mainwaring D E, Harrison S and Fecondo J V 2009 The detection of influenza A and B viruses in clinical specimens using a quartz crystal microbalance *J. Virol. Methods* **162** 14–21
- [226] Li D *et al* 2011 A nanobeads amplified QCM immunosensor for the detection of avian influenza virus H5N1 *Biosens. Bioelectron.* **26** 4146–54
- [227] Wang R and Li Y 2013 Hydrogel based QCM aptasensor for detection of avian influenza virus *Biosens. Bioelectron.* **42** 148–55
- [228] Jiang Y *et al* 2015 SAW sensor for Influenza A virus detection enabled with efficient surface functionalization *Sensors Actuators B* **209** 78–84
- [229] Erofeev A S, Gorelkin P V, Kolesov D V, Kiselev G A, Dubrovin E V and Yaminsky I V 2019 Label-free sensitive detection of influenza virus using PZT discs with a synthetic sialylglycopolymer receptor layer *R. Soc. Open Sci.* **6** 1–13
- [230] Ilic B, Craighead H G, Krylov S, Senaratne W, Ober C and Neuzil P 2004 Attogram detection using nanoelectromechanical oscillators *J. Appl. Phys.* **95** 3694–703
- [231] Migoń D, Wasilewski T and Suchy D 2020 Application of QCM in peptide and protein-based drug product development *Molecules* **25**
- [232] Zhao X *et al* 2012 Protein functionalized ZnO thin film bulk acoustic resonator as an odorant biosensor *Sensors Actuators B* **163** 242–6
- [233] Di Pietrantonio F *et al* 2016 A Shear horizontal surface acoustic wave biosensor for a rapid and specific detection of d-serine *Sensors Actuators B* **226** 1–6
- [234] Ji H F, Thundat T, Dabestani R, Brown G M, Britt P F and Bonnesen P V 2001 Ultrasensitive detection of CrO_4^{2-} using a microcantilever sensor *Anal. Chem.* **73** 1572–6